# STUDIES ON THE PHOTORECEPTORS OF *ANCHOA MITCHILLI* AND *A. HEPSETUS* (ENGRAULIDAE) WITH PARTICULAR REFERENCE TO THE CONES

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Photoreceptors of anchovies *Anchoa mitchilli* and *A. hepsetus* consist of normal rods and two unusual kinds of cones. The latter lie in single vertical rows, and the rods lie between them. Both participate in photomechanical movements, and movement of the cones is closely coordinated with that of pigment cell processes. There are long cones having a cuneate outer segment and short cones having a bilobed outer segment. Long and short (bifid) cones alternate within a row and are staggered between adjacent rows. Both kinds possess calycal processes; long cones have a lateral sac or accessory outer segment.

The long and short cones are associated to form a structure called a cone unit, which consists of the outer segment and ellipsoid of a long cone joined to two outer segment lobes of two adjacent short cones. The lobes of the latter are partly enclosed by the ellipsoid of the long cone. A cone row consists of a row of cone units isolated from each other by processes of the pigment epithelium containing stacks of guanine crystals which form a tapetum. Dorsal and ventral faces of inner segments have contact zones characterized by subsurface cisternae.

Lamellae in the cone outer segments are arranged longitudinally with respect to the cell axis and short and long cone lamellae are perpendicular to each other; lamellae of the rods are transverse. Long cone lamellae are perpendicular to the cone row, and in the central retina are almost horizontal to the long axis of the body. Some vesicular/tubular structures also occur in the cone outer segments.

Outer and inner segments of cones are joined by a broad connecting structure containing a stalk and root portion corresponding to a modified and reduced cilium shaft and centriole, respectively. The rod has a typical connecting stalk. Mitochondria of cone ellipsoids have expanded perimitochondrial spaces between outer and inner membranes.

The organization of the anchovy cones is compared with that of other vertebrates. It is suggested that the cone unit may be a two channel analyser for the detection of plane polarized light and function in conjunction with the overlying reflector of regularly arranged platelets.

## INTRODUCTION

The eyes of fishes display a remarkable diversity of structure, which is now being explored by electron microscopy (Villegas 1961; Engström 1963*a*; Stell 1965; Borwein & Hollenberg 1973; Locket 1971*a*, *b*, 1973). Recent studies on the eyes of anchovies (*Anchoa*) have revealed some new and very unusual features (Fineran & Nicol 1976, 1977). The eye of *A. mitchilli* was examined by Nicol, Arnott & Best (1973) as part of a survey of tapeta lucida in bony fishes; they found that the tapetum contained a white diffusing material subsequently identified as guanine (Zyznar & Nicol 1973). An electron micrograph of the larval eye showed rows of tapetal platelets investing outer segments of photoreceptors which were labelled rods (Nicol *et al.* 1973). It was also noted that the disks (lamellae) of the outer segments ran vertically, i.e., longitudinal to the cell axis. As a result of these initial observations of what appeared to be

unusual features in the tapetum and photoreceptors, we commenced a detailed study of *A. mitchilli* and *A. hepsetus*.

In a preliminary report on the retinal organization of these anchovies (Fineran & Nicol 1976) we demonstrated that the photoreceptors associated with the pigment epithelium system of platelets are cones not rods. Furthermore, we have shown that the cones are grouped into rows which run vertically throughout the retina and that each row is made up of alternately placed long cones and short cones. The short cones were not detected by Nicol *et al.* (1973) and are unusual in that the outer segment is divided into two lobes of equal size. Because of this distinctive feature we have called these cones 'bifid cones'. The longitudinal orientation of lamellae noted by Nicol *et al.* (1973) in long cones of the larval eye has been confirmed for the adult fish. Lamellae of the short cone are also longitudinal and orthogonal to those of the long cone. Another distinctive feature of the cone system is that the long and short cones are united to form a composite structural, and presumably functional, entity which we have called the 'cone unit'. Each cone unit consists of the outer segment of a long cone, and part of its ellipsoid united to two outer segment lobes of the two adjacent short cones.

In the present paper our detailed observations on the organization of the photoreceptors, and especially the cones, are described and discussed in relation to how the photoreceptor might function in association with the pigment epithelium. A full description of the latter has already been published (Fineran & Nicol 1977).

## Materials and methods

Eyes were studied of the bay anchovy *Anchoa mitchilli* Cuv. *et* Val, and the striped anchovy *A. hepsetus* L. caught in the vicinity of Port Aransas, Texas. Fish captured by seining or trawling during daylight hours are designated light adapted (l.a.); those caught by seining at night, dark adapted (d.a.). They were prepared for examination by optical and electron microscopy according to the procedures previously described (Fineran & Nicol 1977). The three dimensional morphology of individual long and short cones, and their association in cone rows in the formation of cone units, were determined from serial sections cut both tangentially and transversely through the retina and from isolated cones in teased preparations. The information from these was used to construct clay models of the cells from which figures 7, 8 and 18 were drawn.

## Observations and results

The appearance of intact eyes, fresh tissues and the general histology of the retina have already been described (Fineran & Nicol 1977).

### (*a*) *Histology of the photoreceptor layers*

A typical transverse horizontal section through a portion of the retina is shown in figure 1, plate 1. The cones form a well-defined layer lying against the external limiting membrane with the rods occupying a broader zone more sclerad. In the horizontal plane the rods and cones appear as distinct radial files with the files of cones alternating with those of the rods (figure 2, plate 1). The files of rods are separated by pigment epithelium cells that occur on the same radii as those of the cones. Each file of rods consists of two to five outer segments lying side by side. In contrast, the files of cones are uniseriate and each cone unit has an elongated, somewhat rectangular, truncate appearance.

## Description of plate 1

Figures 1–5. Optical micrographs of the retina in *Anchoa mitchilli* from epoxy resin embedded material cut between 2–5 μm. Figure 1, unstained section, phase contrast optics. Figures 2–5, sections stained in methylene blue/azur blue. Figures 1–4, light adapted (l.a.) eyes.

Figure 1. Transverse horizontal section through portion of an eye showing the general histology of the retina. Note the vitread compact layer of cones, abutting the outer nuclear layer, and the sclerad broader zone (right) containing alternate rows of rods (light rows) and pigment epithelial cell processes (dark rows). (Magn. × 180.)

Figure 2. Transverse horizontal section at higher magnification showing the sclerad multiseriate partitions of rods and the vitread alternately placed uniseriate rows of cones. Because of the alternate arrangement of cone units in adjacent cone rows and the obliquity of sectioning, some of the cones appear short and discontinuous. (Magn. × 720.)

Figure 3. Tangential section of a l.a. retina cut at slightly different levels through the photoreceptor layer. On the left, the section has passed through the outer segment region of the long cones whereas in the centre and right of the micrograph the cone units have been cut at the level of the short cone outer segments. Note the vertical elongation of the cone units at this level and their separation by a clear space from the associated pigment epithelium cells (compare figure 11, plate 2). The section is orientated so that the cone rows run vertically as in the eye of the living fish. Because cone units of adjacent rows alternate, diagonal patterns of rows appear superimposed on the vertical rows. (Magn. × 840.)

Figure 4. Transverse vertical section cut parallel to a cone row showing the characteristic 'saw-toothed' profile of the cone units in this plane of section. The lobes of the short cone outer segment are clearly visible and are shared between two long cones. Note the dense staining of the short cone inner segment, compared with that of the long cone, and the position of the connecting structure abutting the notch of the outer segment lobes. Owing to the curvature of the retina, the section has passed mainly out of the plane of the inner segment regions on the right of the micrograph. (Magn. × 930.)

Figure 5. Tangential section of a dark adapted (d.a.) eye cut at different levels through the photoreceptor layer. On the far left the section passes through the tapetal layer of the pigment epithelium, near left through the tips of the cone units, in the middle through the outer segment lobes of the short cones, and on the near right through the ellipsoid regions of the cone rows. The top right of the section shows the main layer of rods but the rows of cones are indistinct due to their greatly narrowed myoids at this level (compare figure 16, plate 3). This eye is incompletely dark adapted because scattered rods still occur in the tapetal layer and amongst the cone units. The micrograph has the same orientation as figure 3. (Magn. × 410.)

## Description of plate 2

Figures 9–13. Isolated preparations of photoreceptors from l.a. eyes of *A. mitchilli*. The level of the external limiting membrane is indicated by double-barbed arrows. Nomarsky interference microscopy of retinae treated with EDTA. (All magn. × 1270.)

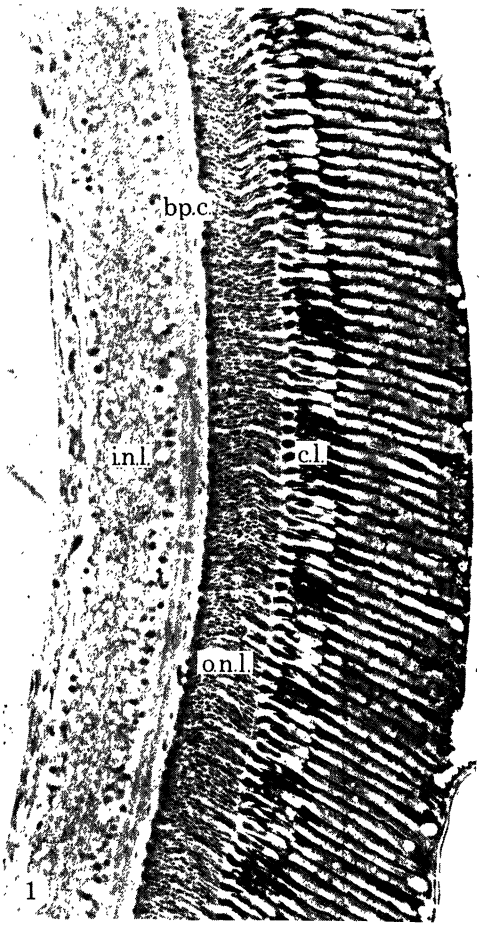
Figure 9. Lateral view of an isolated long cone showing the truncate appearance, in this plane, of the outer segment, and the long ellipsoid and short myoid of the inner segment. Vitread to the level of the external limiting membrane, the cell first narrows and then widens in the nuclear region and narrows again before expanding into the foot.

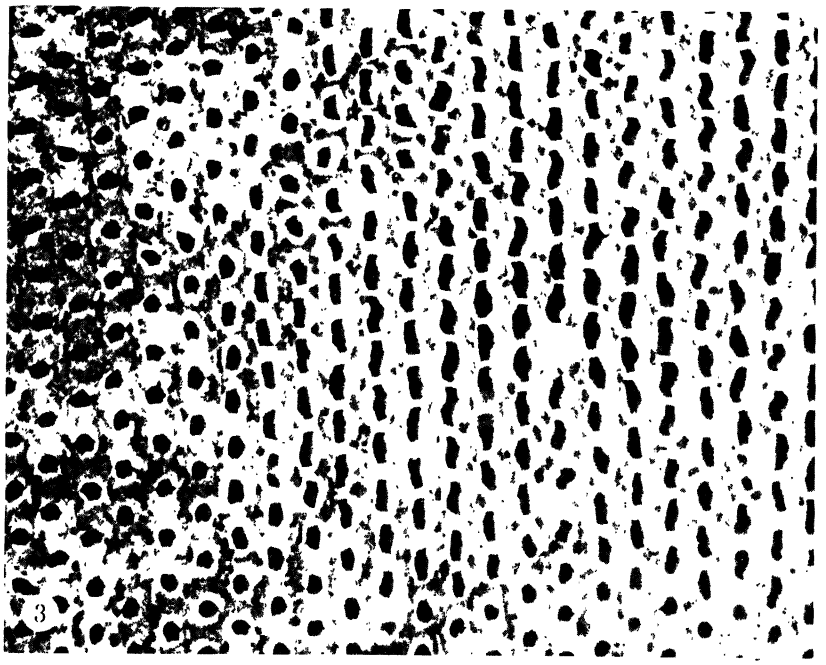
Figure 10. An intact short cone (left), still attached to portion of a long cone, showing clearly the bilobed form of its outer segment. Note the shorter myoid region of the bifid cone compared with that of the long cone.

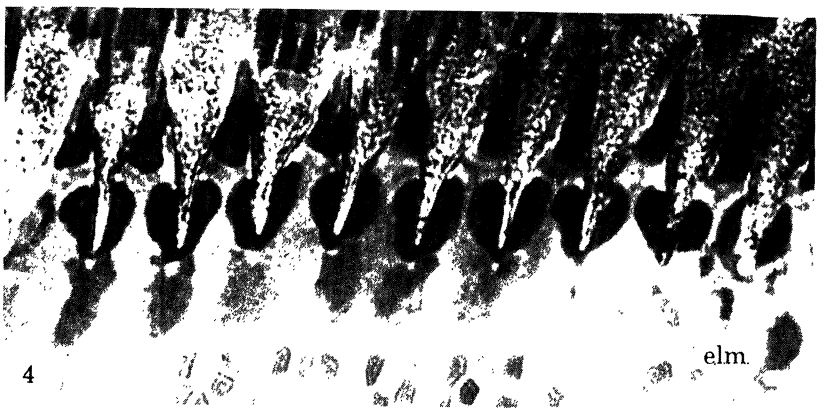
Figure 11. Portion of a short cone (right centre) showing its bilobed outer segment, and various rods. The cylindrical form of the rod outer segment and the short adjoining myoid is well revealed in this preparation.

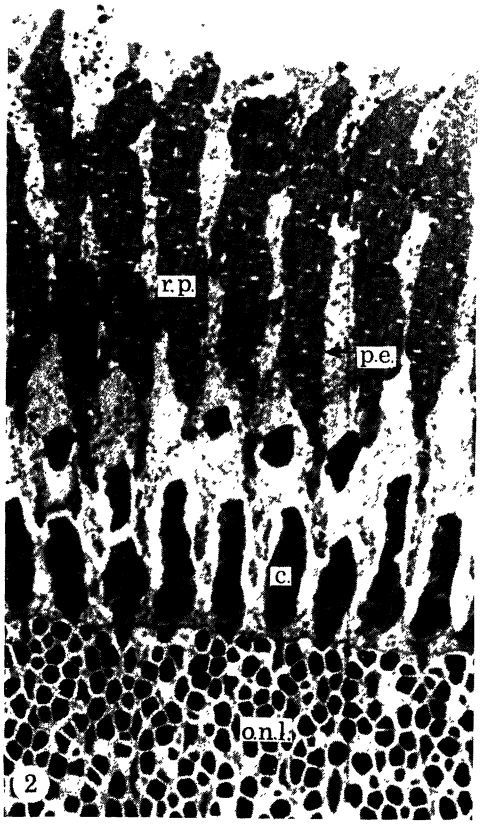
Figure 12. Lateral view of part of a cone row showing the closely linked arrangement and columnar appearance of the cells. As a result of specimen preparation, the outer segments of the long cones are somewhat distorted while those of the short cones are swollen so that they do not show their bilobed character. Note the lateral sac lying alongside two of the long cone outer segments (arrow).

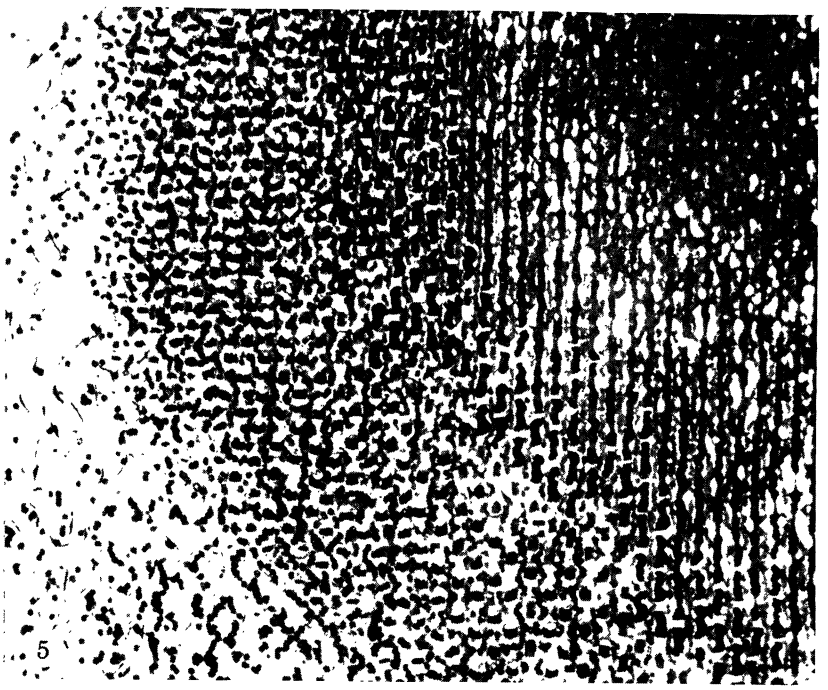
Figure 13. Portion of a cone row showing the alternate sequence of long cones and short cones and the appearance of these cells vitread to the level of the external limiting membrane. The outer segments of the long cones have been damaged during isolation of the cells.




FIGURES 1–5. For description see opposite.

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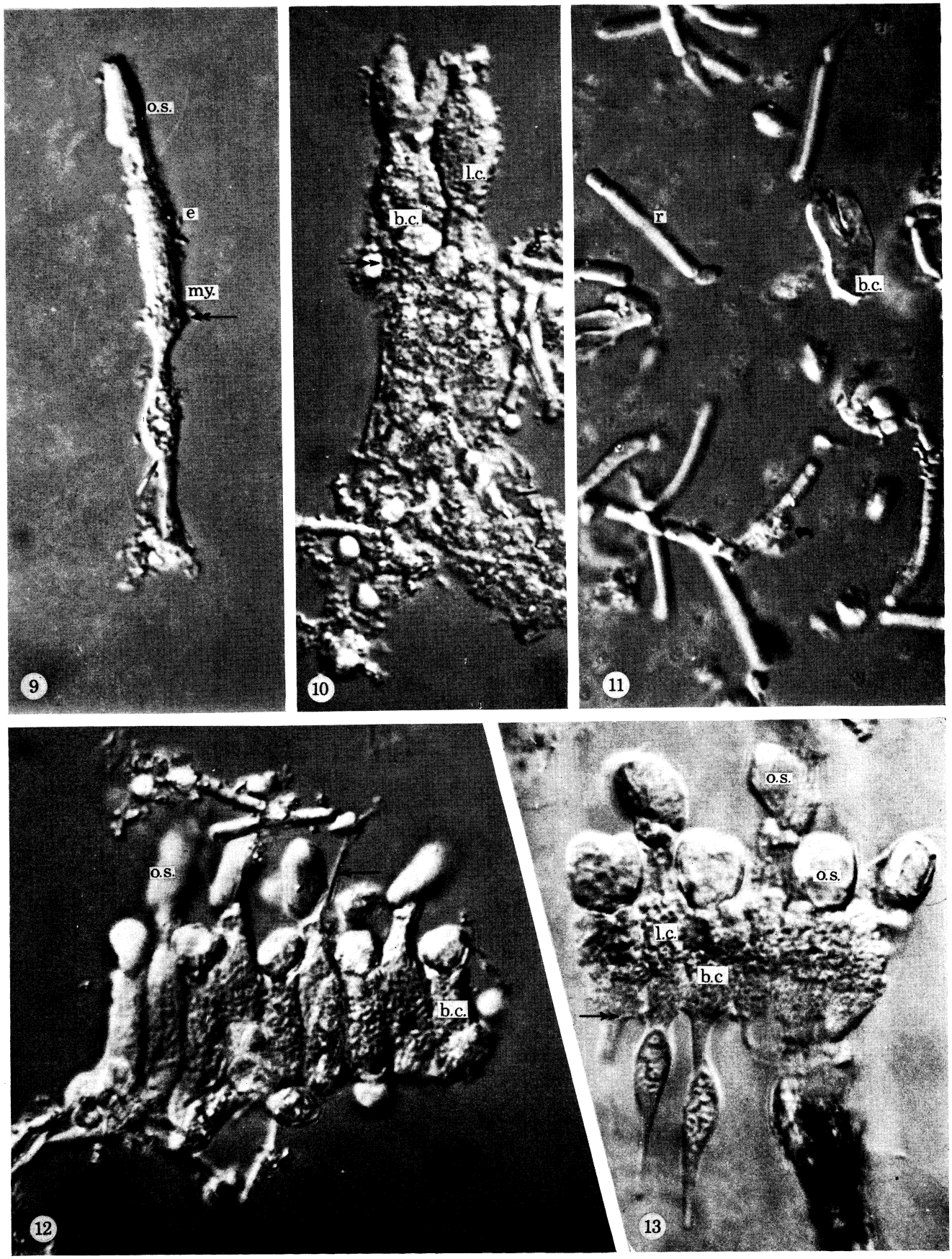
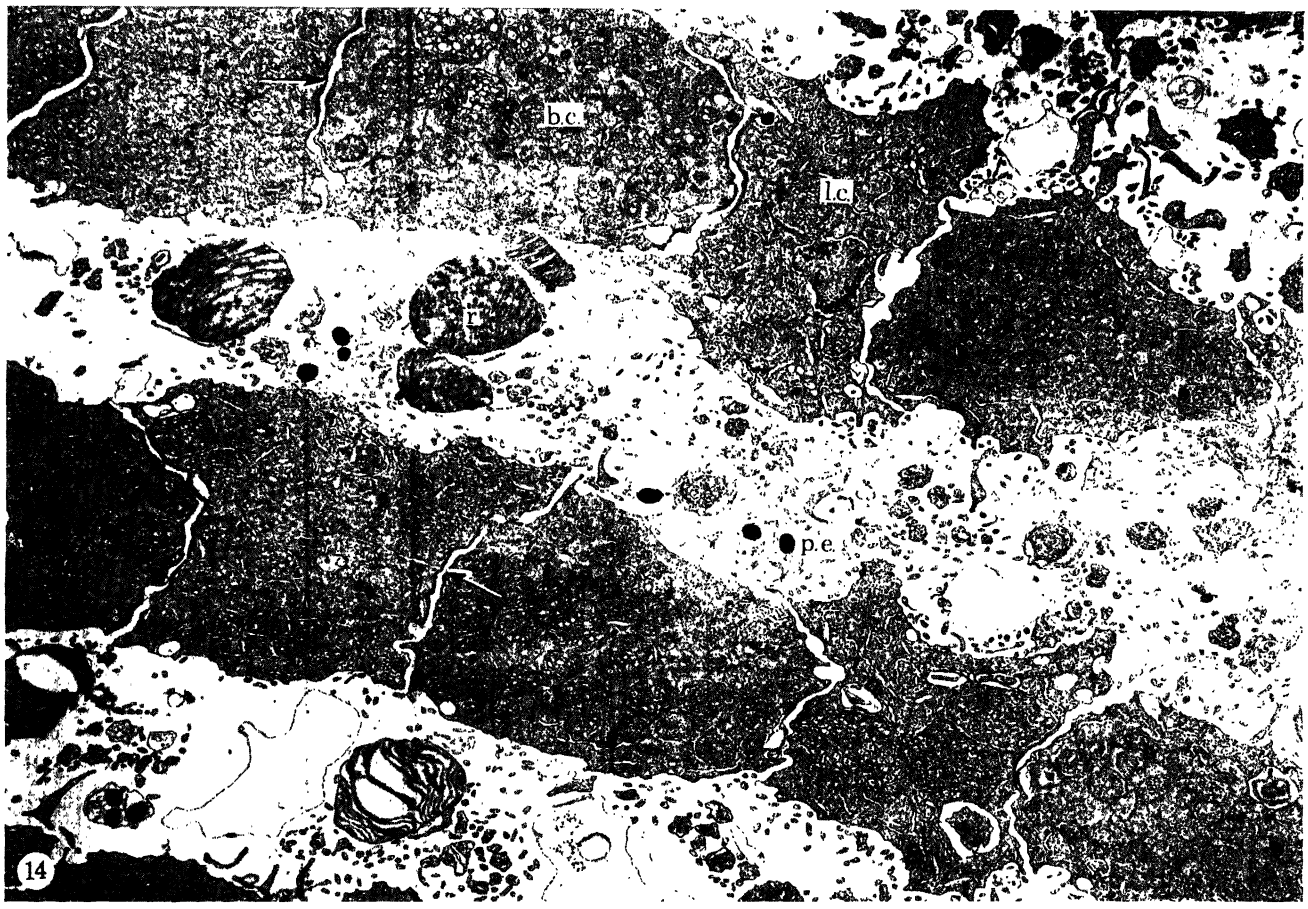
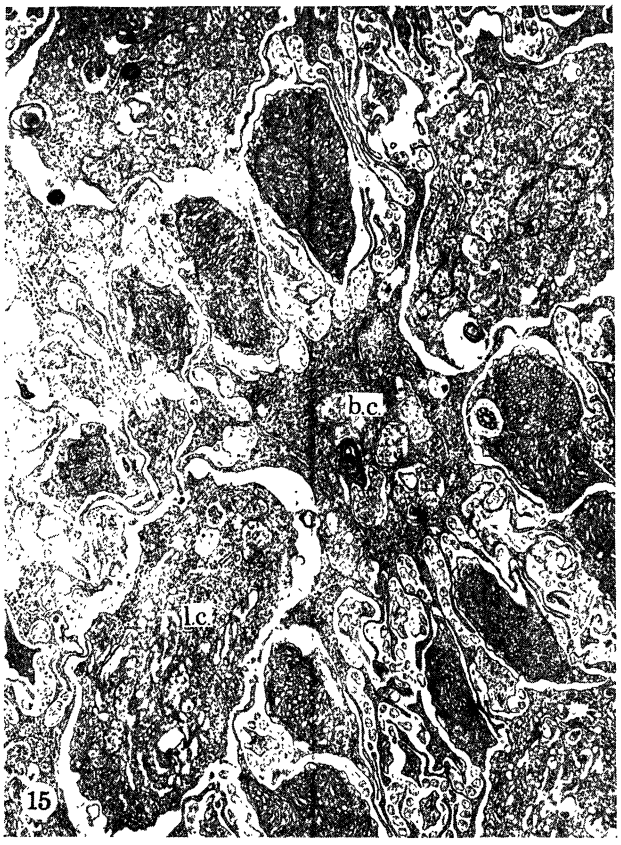
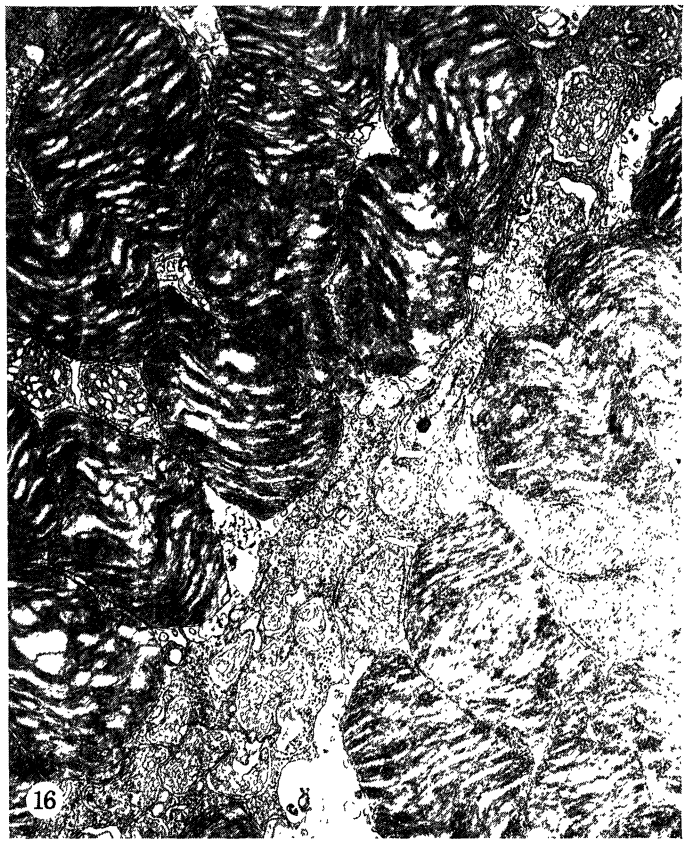



FIGURES 9–13. For description see page 28.




FIGURES 14–16. For description see page 29.

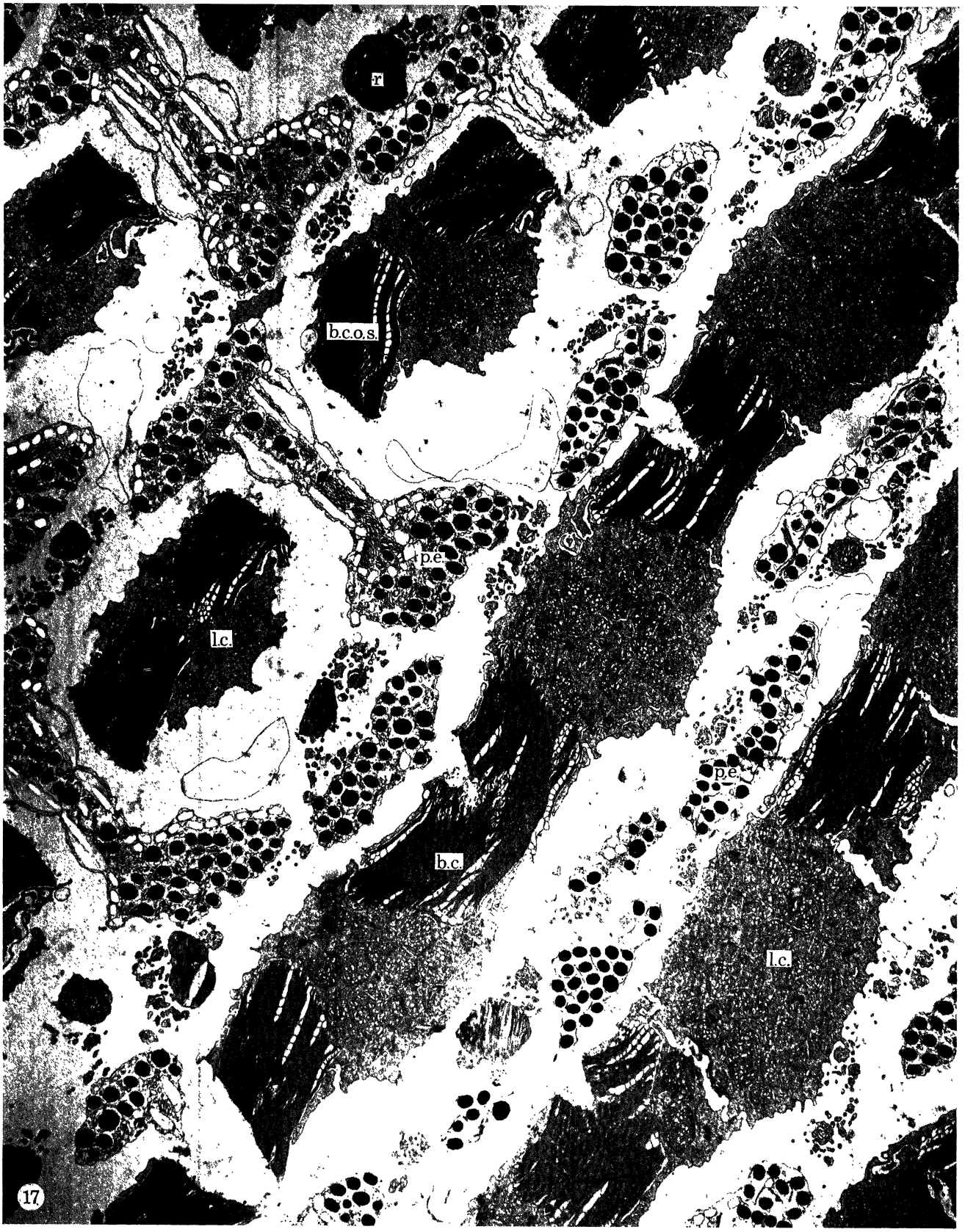



FIGURE 17. For description see opposite.

Tangential sections of the retina reveal that the files seen in the horizontal plane represent rows that extend dorsoventrally throughout the retina (figures 3 and 5, plate 1). In vertical section the files show as radial partitions of photoreceptors and pigment epithelium cells. However, because of the curvature of the retina and the thinness of the partitions, especially those of the cones, it is difficult to cut sections in this plane without some degree of obliquity. When a cone row is sectioned sufficiently parallel throughout a number of cells the cones typically show a 'saw-tooth' shaped appearance with the 'teeth' extending about two-thirds the radial depth of the partition (figure 4, plate 1).

In the d.a. eye the position of the photoreceptor layer is the reverse of the l.a. state (figure 6, see also figure 2, plate 1, Fineran & Nicol 1977). During photomechanical movements leading to the d.a. condition the rod myoids contract, whereas those of the cones lengthen pushing the ellipsoids and outer segments sclerad generally beyond the level of the rod outer segments (figure 5, plate 1).

The radial displacement of photoreceptors and pigment epithelium cells of *Anchoa* (figure 6) is typical of the kind of photomechanical movement shown by the eyes of other fishes (see for example, Arnott, Best, Ito & Nicol 1974; Fineran & Nicol 1974). However, the changes are complicated by the linked movement of the cones throughout a row and the integrated displacement of the outer segments with the interdigitating processes of the pigment epithelium. The movement of the rods is not as synchronous and the outer segments remain somewhat scattered (figure 5, plate 1; figure 19, plate 5).

Typical dimensions of the photoreceptor layers of l.a. and d.a. eyes are given in table 1. A diagram showing the relative positions of the rods and cones in the different photoadapted states of the eye is shown in figure 6.

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## Description of plate 3

Figure 14. Tangential section of the retina from a l.a. eye showing cone rows cut transversely through their inner segment regions. On the left the section passes mainly through the ellipsoids whereas on the right the cones have been cut more towards the scleral level of the myoids. The long cones have concave dorsal and ventral faces abutting convex faces of adjacent short cones in the same row. Myoid folds are just beginning to appear on both cones at top right. Numerous closely packed mitochondria fill the ellipsoids and between the cones at this level is a contact zone (arrows). The space between the cone rows contains numerous microvillus-like processes of the Müller cells and rod inner segments. A few rod outer segments and vitread tips of pigment epithelium cell processes are also included. (Magn. × 7000.)

Figure 15. Portion of a cone row near the external limiting membrane cut transversely showing the characteristic outline of the long and short cones. Note the well developed myoid folds of the long cone in the d.a. condition and the numerous microvillus-like processes of the Müller cells between the folds. (Magn. × 12300.)

Figure 16. Portion of a cone row from a d.a. eye showing the narrow partition amongst the rods formed by the elongated myoid region. Note the close packing of the rod outer segments in the d.a. condition. (Magn. × 15100.)

## Description of plate 4

Figure 17. Low power electron micrograph of a tangential section from the retina of a l.a. eye showing cone rows cut at the level of the outer segment of the short bifid cones. The section passes slightly deeper vitreally across the successive rows (from top left to bottom right); the top two rows reveal separate outer segment lobes of the short cones cut transversely whereas in the lower rows the transversely cut lobes of adjacent cone units are continuous. Where the vitread end of the notch between the lobes has been cut obliquely this is indicated by an arrow. In the top two rows of cones, note the H-shape form of the long cone ellipsoid in transverse section and, especially in the second row, its asymmetrical development. Wide ventricular spaces separate the cone units from the associated pigment epithelium cell processes at this level. Between the rows of cones are rods that mainly lie in groups opposite the vitread processes of the pigment epithelium; at this level myoid and ellipsoid portions of the rods are chiefly visible. (Magn. × 6100.)

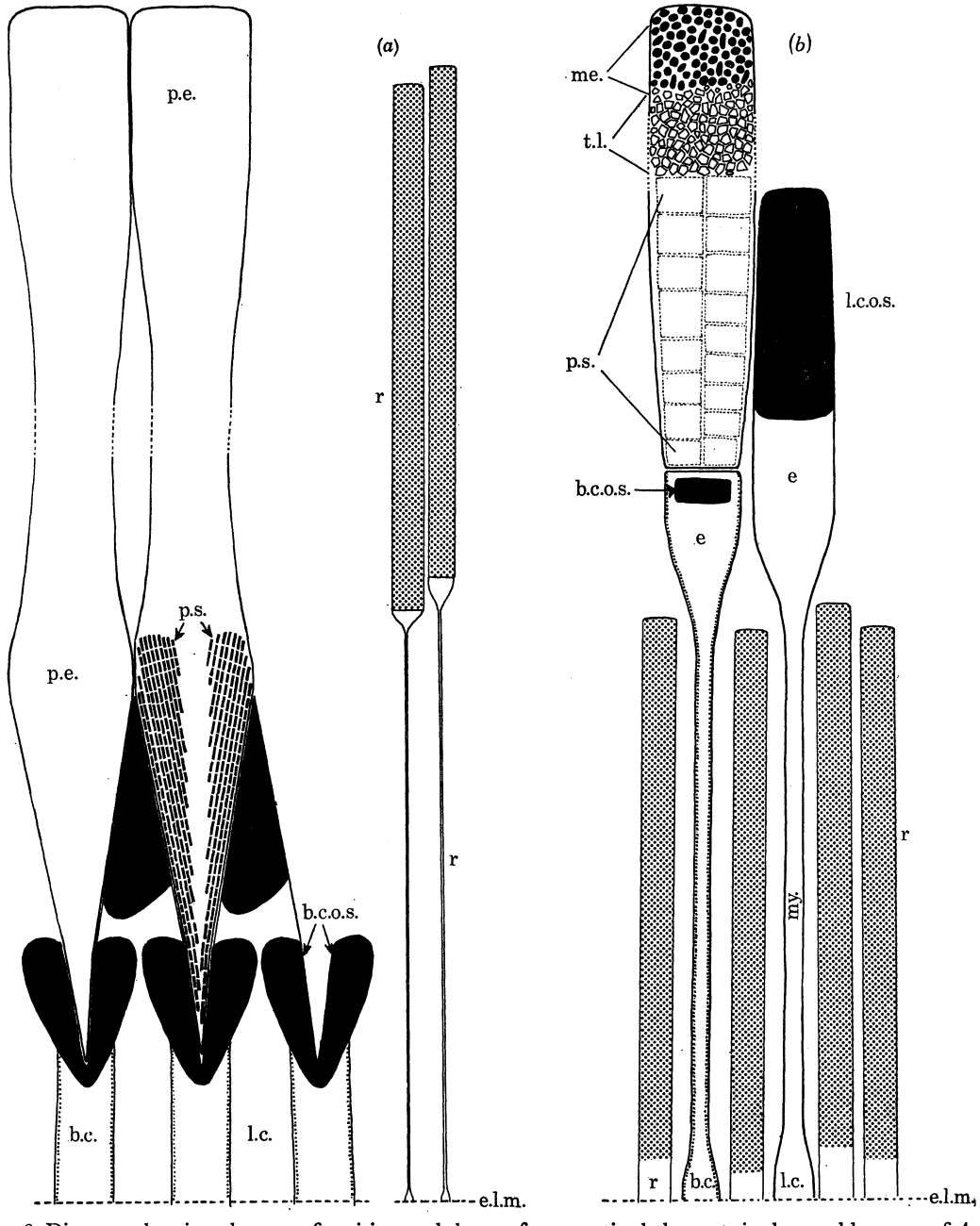



FIGURE 6. Diagram showing changes of position and shape of some retinal elements in d.a. and l.a. eyes of *A. mitchilli* and *A. hepsetus*. Cone outer segments are shown as solid black areas; rod outer segments have dotted shading. (*a*) l.a. eye. Portion of a cone row and its subtending interdigitating pigment epithelium cells in transverse vertical view. Two rods from the rod partition between the cone rows are situated on the right. (*b*) d.a. eye. The position of the cones and rods is reversed relative to that of the l.a. condition. The cone rows are shown in transverse horizontal view with the contracted partitions of rods lying between. The short cone, left, is drawn as if cut medianly through the notch of the outer segment hence only the small portion of the outer segment connecting the lobes is depicted. Immediately scleral to the short cone is the associated pigment epithelium cell with its melanosomes and tapetal crystallites in two layers and a stack of platelets. The platelet stack is shown in dotted outline as it lies out of the median plane. The long cone, right, is shown as if cut medianly.

TABLE 1. DIMENSIONS OF PHOTORECEPTOR LAYERS AND CONES IN CENTRAL FUNDUS SCLERAD TO EXTERNAL LIMITING MEMBRANE

| | | l.a./μm | d.a./μm |
|---|---|---|---|
| Radial extent of | rod layer | 100 | 40 |
| | cone layer | 45 | 85 |
| | cone unit | 30 | |
| | long cone | 43–45 | |
| | short cone | 24 | |
| | long cone outer segment | 18 | |
| | short cone outer segment | 12 | |
| Vertical distance between tips of cone units in same row | | 9–11 | |
| Distance between cone rows at level of long cone outer segment | | 5 | |
| Lateral diameter of cone unit at level of short cone outer segment | | 4–5 | |
| Dorso-ventral diameter of cone unit at its base | | 8–10 | |

### (*b*) *Morphology of photoreceptors*

The retinae of *A. mitchilli* and *A. hepsetus* contain long cones and short cones which have a bilobed outer segment (figures 7 and 8). At the margins of the retina the long cones show some forms transitional towards those more typical of vertebrate eyes.

#### (i) *Long cones*

A long cone has a truncate outer segment joined to a long ellipsoid and a myoid (figure 9, plate 2). In l.a. eyes the myoid is very short but in the d.a. state it is greatly elongated radially and narrow laterally. Vitread to the myoid the cell narrows and then expands in the nuclear region and narrows again near the foot (figure 13, plate 2).

Externally, the long cone shows three distinct regions termed, for convenience, outer, middle and inner (figure 7). In l.a. eyes the outer portion occupies almost half the length and has a cuneate or wedge-shaped form with the distal edge of the wedge orientated horizontally and perpendicularly to the axis of the cone row in which the cell lies. The lateral sides of the wedge, facing adjacent cone rows, are parallel except at the tip of the cone where they begin to converge to give the margins of the cell a slightly rounded appearance, and the vitread end of the cuneate region has a square 'base' in cross section (figure 7).

The cuneate region contains the outer segment and distal portion of the ellipsoid. A single lateral sac (Fineran & Nicol 1974) projects from the vitread end of the outer segment on its temporal face and extends sclerad approximately two-thirds the way along the face. The lateral sac tapers gradually and is connected to the outer segment throughout most of its length.

A series of calycal processes arise from the scleral end of the ellipsoid and run alongside both lateral faces of the outer segment (figure 7). There are usually between five to seven processes on the temporal face, divided by the lateral sac, and up to eight on the nasal face. The longest calycal processes are those near the middle, and those on either side of the median position become progressively shorter. Although the calycal processes lie close to the outer segment there appears to be no direct connection with it. In the l.a. eye, they are continuous with low ridges on the ellipsoid (figure 17, plate 4).

The middle region of the long cone is indented (figure 7; figure 4, plate 1). The shape results from two pocket-like cavities on the opposite dorsal and ventral sides of the cell that accommodate the outer segment lobes of the short cones (figure 4). The inner dorsal and ventral faces of the pockets are flat or gently curved (figure 17), and the vertical sides of the pockets are

parallel. As a consequence of the presence of the pockets, the middle region has an approximate H-shaped appearance in transverse section (figure 17; see also fig. 36, pl. 10 in Fineran & Nicol, 1977). Over the length of the middle region, the lateral diameter of the cell becomes progressively reduced and at the vitread end of the pockets it is about half that of the outer segment region (figure 3, plate 1).

The inner region of the long cone contains the remainder of the ellipsoid and the myoid portions of the cell. In the l.a. eye this portion of the ellipsoid has the same horizontal and vertical diameter. In the myoid region there are longitudinally orientated folds that radiate for varying distances from the cell (figure 7; figure 16, plate 3).

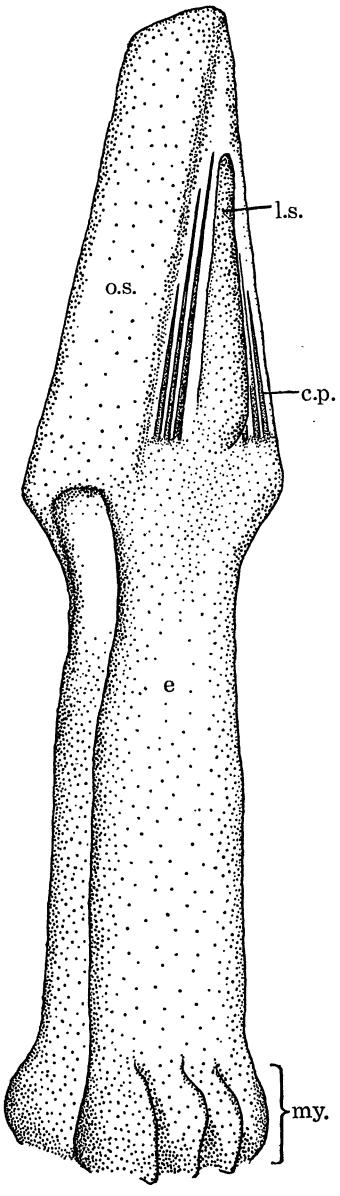



FIGURE 7. Three dimensional representation of the morphology of the photoreceptor layer portion of a long cone as seen in oblique view. L.a. state.

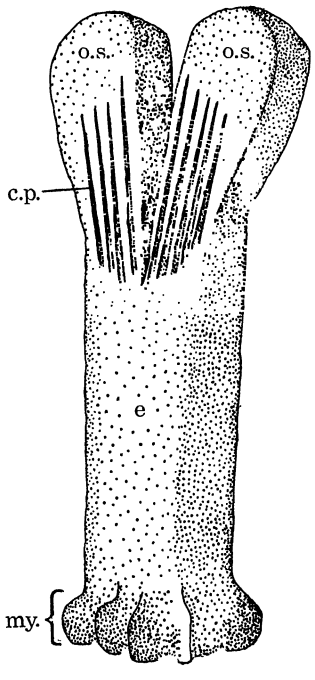



FIGURE 8. Three dimensional representation of the morphology of the photoreceptor layer portion of a short bifid cone as seen in slightly oblique view. L.a. state.

In the d.a. state the inner region of the long cone is altered in shape as a result of photomechanical movements. It becomes greatly elongated through extension of the myoid and possibly also to a small extent by elongation of the ellipsoid. There is a decrease in the lateral diameter of the inner segment with a corresponding, but slighter, increase in the cell's vertical diameter (figure 16). Near the external limiting membrane the myoid increases in size and has a more uniform diameter. The myoid folds in this region are often well developed in the d.a. eye (figure 15, plate 3).

(ii) *Short cones*

The nuclear and foot regions of the cell vitread to the external limiting membrane are similar to those of the long cone, except that the nuclear region is usually situated slightly more vitread (figure 13, plate 2). The portion of the cell lying within the photoreceptor layer consists of an inner segment of normal appearance and an outer segment which is unusual in consisting of two lobes arranged approximately in the form of a V. The lobes are orientated parallel to the cone row and therefore vertical to the body axis of the fish (figure 4, plate 1; figures 10 and 11, plate 2). Figure 8 depicts the morphology of the outer and inner segment regions of the short bifid cone based on examination of thin sections and isolated cells.

The inner dorsal and ventral faces of the lobes are flat and lie at an angle of between 10–12°, corresponding to that of the interdigitating process of the pigment epithelium cell (Fineran & Nicol 1977). The lateral faces of the lobes are parallel and also flat, and the outer lobes and ventral sides are strongly curved radially. As a consequence, the outer segment has a deeply incised heart-shaped profile when seen in lateral view (figure 4; figure 48, plate 15). There is some alteration in the shape of the lobes in the different photoadapted states of the eye. In the l.a. condition the lobes are compressed laterally and drawn out dorso-ventrally, giving them a rectangular appearance in cross-section (figure 17, plate 4; figure 29, plate 9). In the d.a. state the lobes are more compressed dorso-ventrally and expanded laterally (figure 20, plate 6). The faces of the dorsal and ventral sides of the outer segment lobes, respectively, are usually gently curved or flat with rounded edges in the l.a. eye (figure 17), and become flatter in the d.a. state (figure 20).

Calycal processes, similar in form to those of the long cone, arise from the outer end of the ellipsoid and extend alongside the lateral faces of both outer segment lobes (figure 8). There is no lateral sac.

The ellipsoid has a short stout columnar form, rectangular in cross section (figure 8; figure 14, plate 3). The lateral faces are parallel and generally flat whereas the dorsal and ventral faces are convex (figures 14 and 15, plate 3). The myoid in the l.a. state is shorter than that of the long cone (figure 18) and half the diameter of the ellipsoid. Myoid folds are similar in development to those of the long cone.

In the d.a. state the inner segment is greatly elongated radially but its form is basically the same as in the l.a. eye (figure 16, plate 3).

(iii) *Rods*

Figure 11, plate 2, shows the morphology of rods isolated from a l.a. eye. The cylindrical outer segment has a diameter of 1.5 μm and an average length of 20 μm. The ellipsoid is short but the myoid is long and greatly narrowed in the l.a. state (figure 6). A lateral sac is absent; calycal processes were not found.

### (*c*) *Arrangement of photoreceptors*

The photoreceptors of *A. mitchilli* and *A. hepsetus* show a regular arrangement into rows that run in a vertical plane relative to the body axis of the fish throughout the eye (figures 1–5, plate 1).

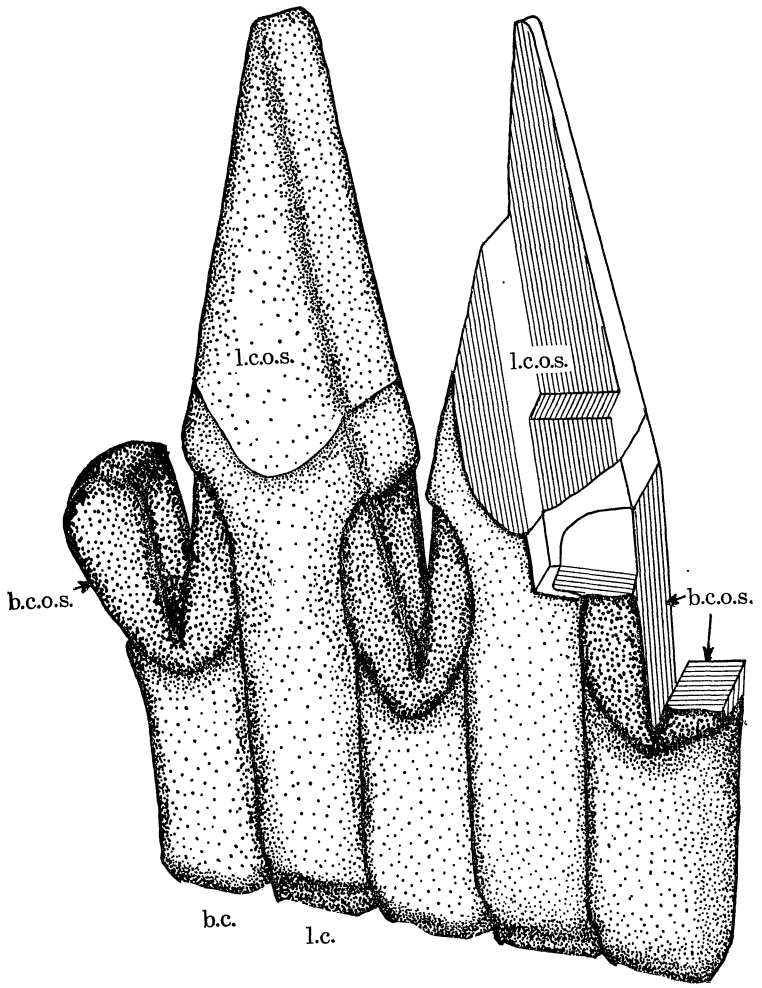



FIGURE 18. Portion of a cone row from a l.a. eye showing the three-dimensional relationship between long cones and short bifid cones in their formation of cone units. The cut-away areas on the right illustrate the orientation of outer segment lamellae of both cones within a cone unit and the manner in which the bifid cone lobes press into the long cone ellipsoid. In the middle short cone only that portion of the lobes outside the pockets is visible. A complete lobe is shown in the short cone on the left. Note the columnar appearance of the inner segments and the short myoid regions, especially that of the short cone. Calycal processes and myoid folds have been omitted from the drawing in both cones and also the lateral sac of the long cone.

#### (i) *Cones*

The cones form uniseriate rows of alternately placed long and short cones (figure 14). Their inner segments lie side by side (figures 12 and 13, plate 2) so that the convex faces of the short cones fit into the corresponding dorsal and ventral concave faces of the long cones. The inner segments remain closely linked during photomechanical movements and often remain intact in isolated cell preparations (figures 12 and 13).

The outer segments of both cones are intimately associated and form a cone unit (Fineran &

Nicol 1976). Each cone unit consists of a central axis formed by the long cone outer segment and ellipsoid united dorsally and ventrally to an outer segment lobe from each of the adjacent short cones (figures 12 and 13). The cone unit is a composite structure containing portions of three cones combined in such a way that the entity has a distinctive cuneate form (figure 18; figure 24, plate 8). The converging faces of the cone unit are perpendicular to the cone row. Cone units are best visualized in transverse vertical sections of the retina cut parallel to a cone row (figure 4). They are rarely isolated intact as the outer segment of the long row usually becomes detached or the lobes of the short cones are obscured (figures 12 and 13). Typical dimensions of a cone unit and the distances between other units and cone rows are given in table 1.

The cone units of adjacent rows show an alternate arrangement (figure 3; figure 17; figure 19, plate 5). Sections through long cone outer segments show an almost hexagonal pattern (figure 19). The vertical alignment of the cone unit is shown best in l.a. eyes in tangential sections of the retina through the bifid cones outer segments (figure 17). In the fundus, the cone units lie perpendicular to the plane of the retina but at the periphery they are more inclined.

(ii) *Rods*

Rods are arranged into vertical groups, 4–6 cells wide, that run throughout the eye between the rows of cones (figures 1 and 2; figure 20, plate 6). The rods show no precise association with each other or with the cones but rather fill the space between the rows of cones. Rods are mostly distributed randomly along the length of a partition but in l.a. eyes at the level of the cone units there is a tendency for their myoid and ellipsoid regions to be grouped opposite the vitread processes of the pigment epithelium (figure 17). In l.a. eyes the outer segments of the rods mainly lie sclerad to the tips of the cone units with their elongated myoids running between the rows of cones (figure 1); in the d.a. condition rod outer segments usually reach the level of the short cone outer segment (figure 20). In the d.a. state the outer segments of the rods are closely packed between the cone rows (figure 16), but in the l.a. condition the packing is less dense.

### (*d*) *Ultrastructure of the photoreceptors*

(i) *Long cones*

(1) *Outer segment.* Most of the long cone outer segment is cuneate like the cone unit of which it forms part, but the inner region is blunter where it converges on the connecting structure (figure 22, plate 7).

Except at the periphery of the retina, the photosensitive lamellae of the outer segment have a longitudinal orientation, with respect to the axis of the cell (figure 18; figures 24 and 25, plate 8). The lamellae, therefore, become progressively shorter vitread along the dorsal face. Near the vitread end of the outer segment the lamellae gently curve towards the connecting structure (figure 37, plate 11; figure 41, plate 13), and they become progressively fewer on approaching the stalk (figure 22, plate 7). The lamellae are continuous with each other and the cell membrane as in a typical cone; the ends are closed along the dorsal face of the outer segment (figure 25, plate 8). Transverse sections of the outer segment reveal lamellae with closed ends on the temporal face that project into the intracellular space formed by the overarching of the cell membrane, and open ends on the naso-lateral face (figure 26, plate 8). This configuration of lamellae within a cone is constant among long cones throughout the retina, except at the periphery of the eye.

In l.a. eyes the lamellae form close parallel sheets. The width of a lamella and inter-lamella space is about the same (figure 21, plate 6). The parallel array of lamellae is sometimes interrupted by a local buckling of membranes, particularly in d.a. eyes where there may occur both lateral and radial compression of the outer segment (figure 19, plate 5), apparently caused by the sclerad movement of the cones during retinomotor changes. The lamella stacks may also be distorted occasionally by the formation of vesicles and/or tubules (figures 19 and 25), which contain ground substance resembling that found between the lamellae.

A system of vesicles and/or tubules different from those noted within the lamella stacks occurs regularly along the nasal margin of the outer segment. These 'marginal vesicles,' as they appear in section, are usually more frequent in the short (figure 44, plate 12) than in the long cone. The contents of the marginal vesicles are normally more electron dense than the ground substance between lamellae, with which they are continuous. A central core can be detected in some of the marginal vesicles. The system is usually more extensively developed towards the vitread end of the outer segment.

Whereas the lamellae in the long cone are usually arranged longitudinally throughout the fundus, the situation is different at the margin of the eye (figures 30 and 31, plate 10). Here the cones nearest the margin have transversely orientated lamellae. The ends of the lamellae are closed along the temporal, ventral and dorsal margins but open anteriorly. The lateral sac lies in a median position. With increasing distance from the margin, over a sequence of about 6–10 cones, the orientation of lamellae progressively changes so that they eventually come to lie parallel to the ventral side of the cone. The first indication of this change is a gentle curving of lamellae vitread, usually throughout the length of the outer segment, and this is followed by the sclerad lamellae becoming progressively longitudinally orientated and last of all the most

## Description of plate 5

Figure 19. Tangential section through the retina of a d.a. eye showing the appearance of the cone units at the level of the long cone outer segment about midway along its length. The micrograph is orientated so that the cone rows run vertically with the cone units of adjacent rows alternating. The outer segment lamellae of the long cones and the associated platelets of the pigment epithelium cells have a horizontal orientation and the lateral sacs (arrows) all lie on the temporal (left) side of the cones. The lamellae are a little distorted due to lateral compression of the outer segment on dark adaptation of the eye. Also the organization of the associated pigment epithelium cells at this level is modified in comparison with the l.a. condition by the disappearance of lateral lobes and the scleral withdrawal of melanosomes to the basal region of the cell. A few rod outer segments extend to the level of the long cone outer segment and lie between the otherwise closely opposed rows of cones. (Magn. ×4000.)

## Description of plate 6

Figure 20. Tangential section through the retina of a d.a. eye showing the appearance of the cone units at the level of the short cone outer segment lobes. The micrograph is orientated so that the cone rows run vertically as in the eye of the living fish. Compared with the l.a. condition of the retina (see figure 17, plate 4), the lobes of the short cones are wider laterally and dorsoventrally compressed which has resulted in buckling of the lamellae. The ellipsoid portion of the long cone situated between the short cone lobes (arrows) is narrower than in the l.a. state. Some rod outer segments are present at this level and form mainly single rows between those of the cones. Note the disappearance of the ventricular space from around the cone units and the absence of lateral lobes in the pigment epithelium cells compared with the l.a. eye. (Magn. ×4800.)

Figure 21. A comparison between rods and cones of the lamella/disk thickness and spacing in different areas of the same micrograph with the same optical enlargement. (*a*) shows the lamellae in portion of a long cone outer segment and (*b*) the outer segment disks of a rod. In the cone the lamellae and interlamella cytoplasmic space are the same thickness. In the rod the disks are thicker than the lamellae of the cones but the inter-disk space is the same. (Both magn. ×53000.)

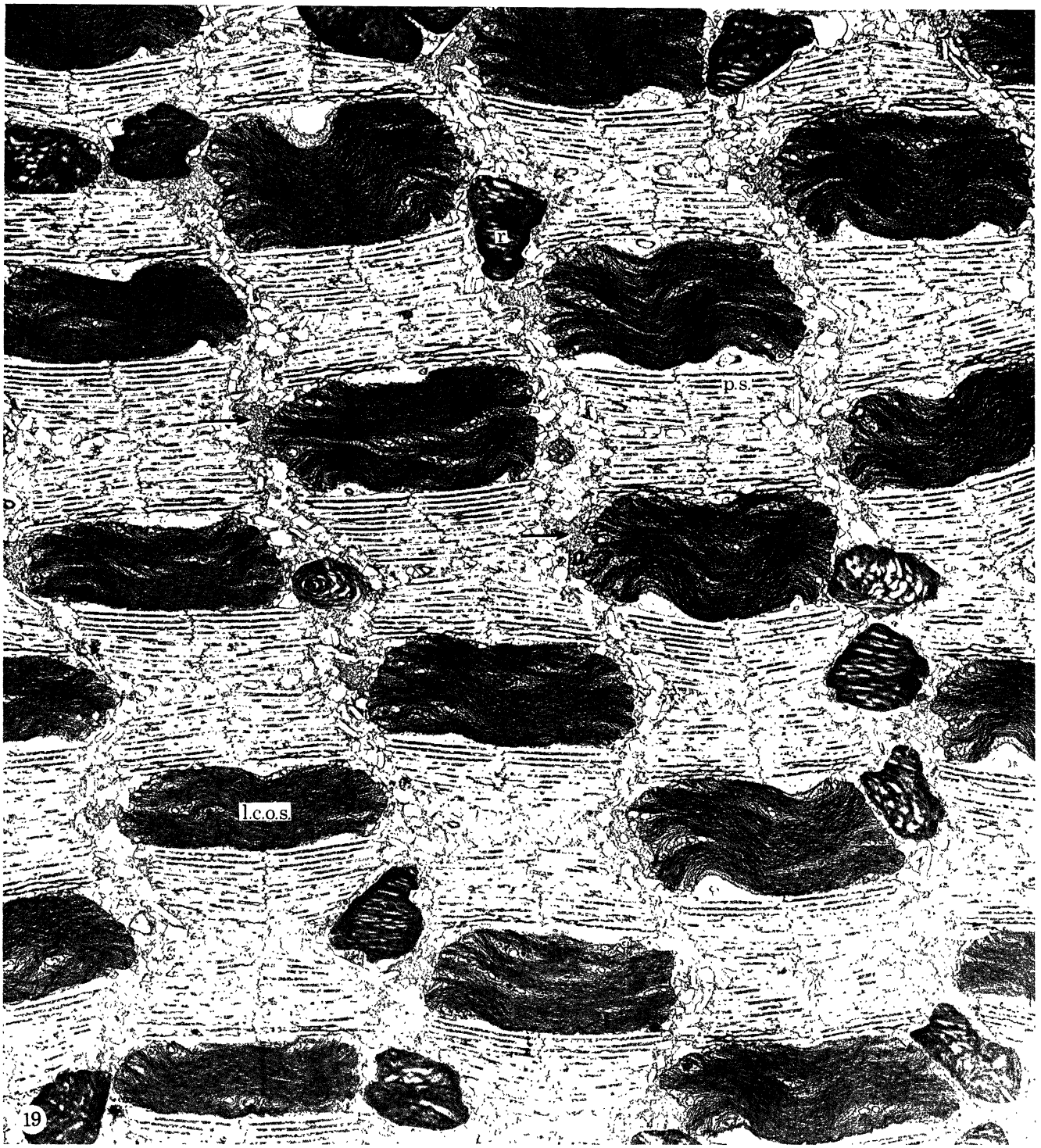
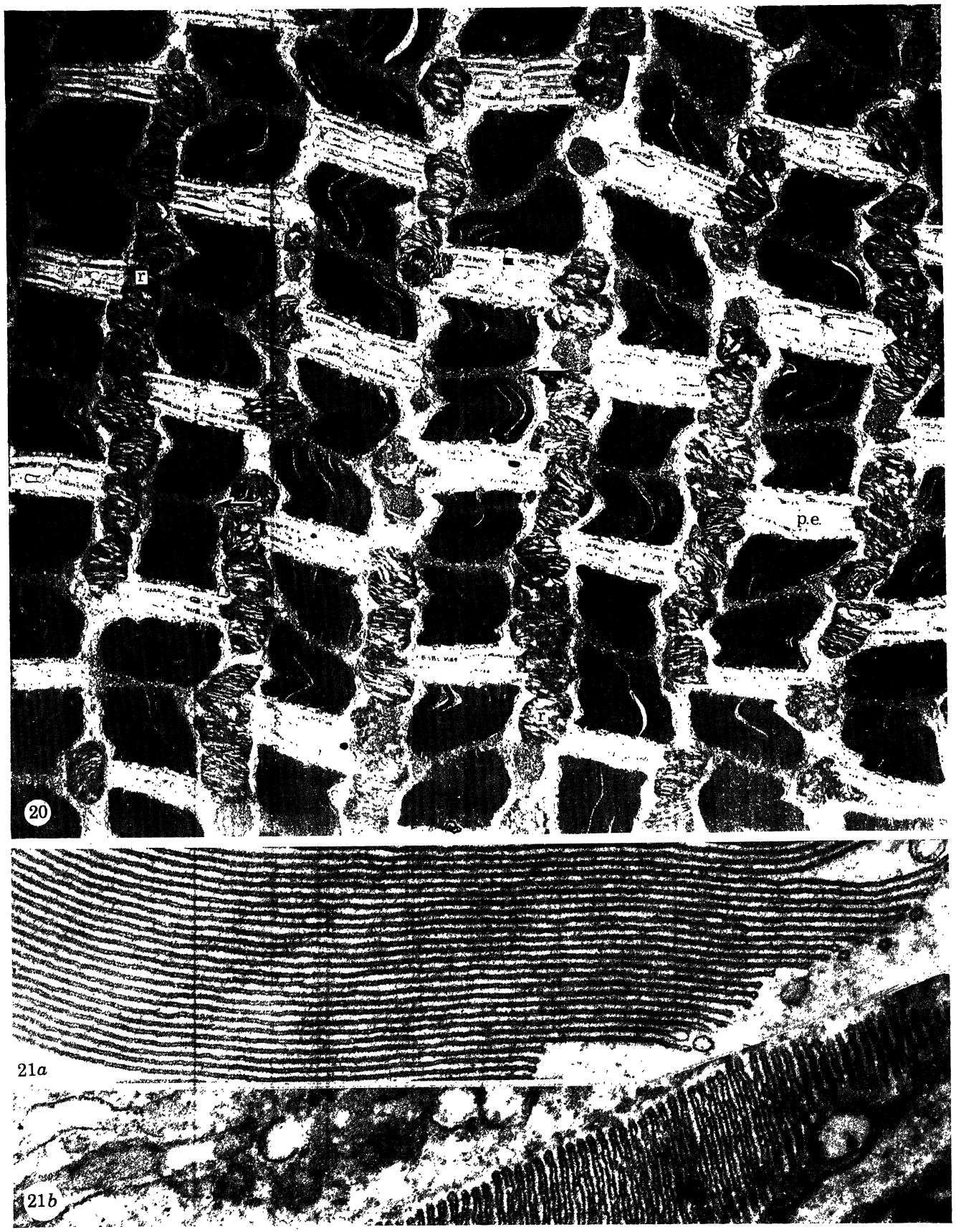
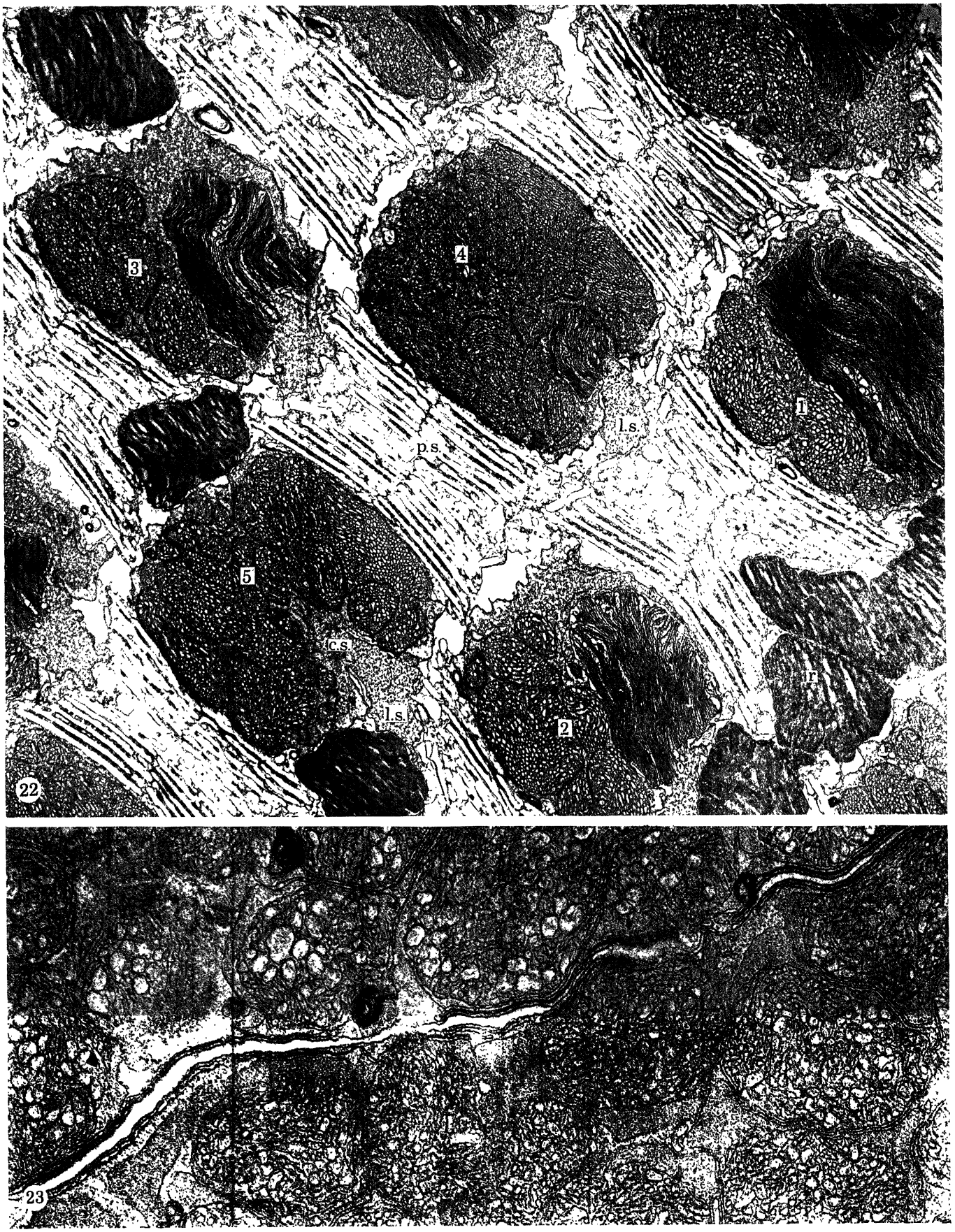



FIGURE 19. For description see opposite.

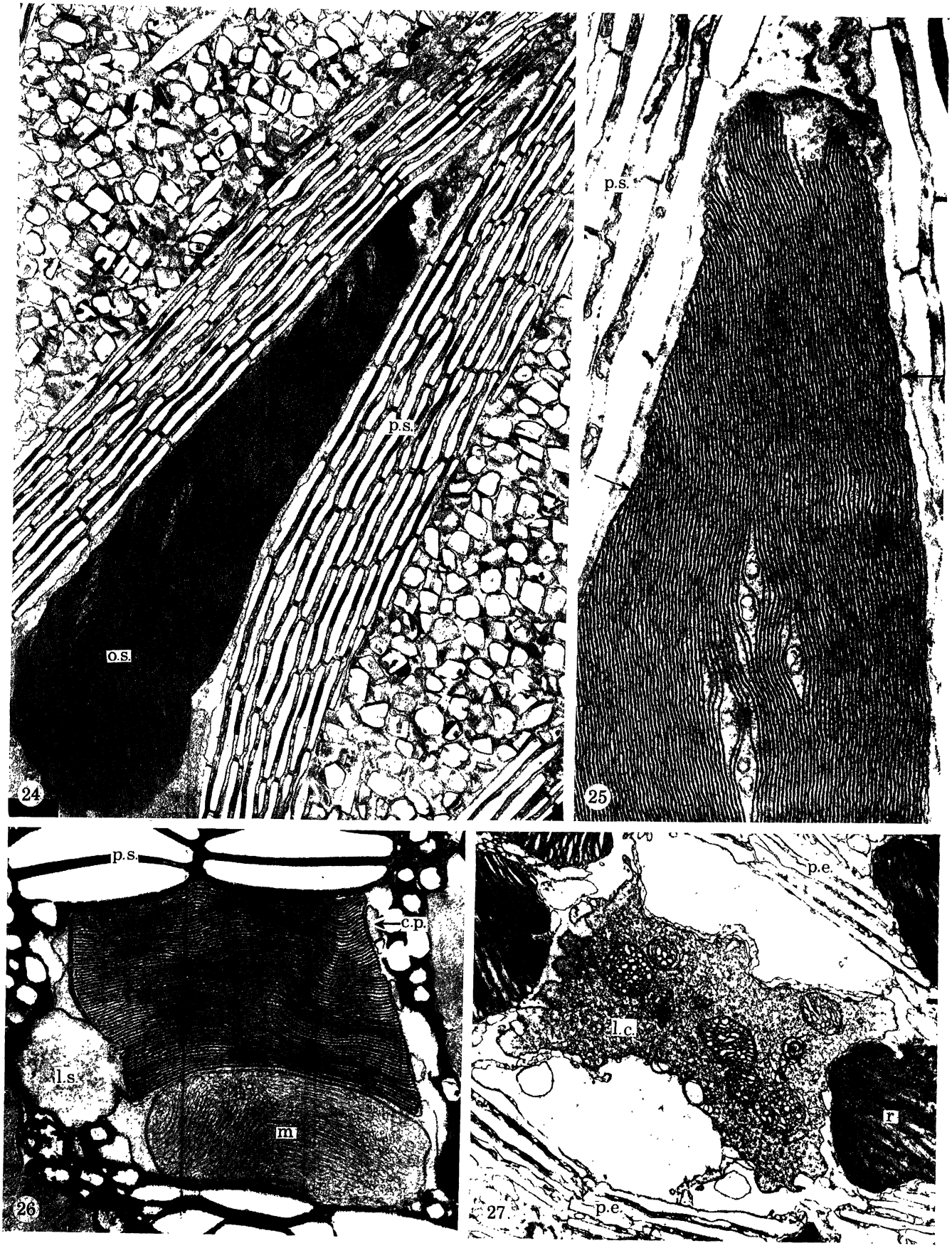
(*Facing p.* 36)




FIGURES 20–21. For description see page 36.




FIGURES 22–23. For description see page 37.




FIGURES 24–27. For description see opposite.

vitread lamellae. Such a change in orientation results in lamellae having open ends on the naso-lateral face and closed margins on the dorsal face. The shift in orientation of photosensitive lamellae is accompanied by changes in the associated pigment epithelium cells involving an alteration in the relative abundance of melanosomes and tapetal material (Fineran & Nicol 1977).

(2) *Inner segment.* The connecting structure between outer and inner segments consists of a short wide cylindrical stalk (figure 22, plate 7; figures **34**, **36** and **37**, plate 11), lying in a tube formed by infolding of the cell membrane, and a root portion. The extracellular space surrounding the stalk contains a cylinder of finely granular material separated from the remaining electron transparent contents (figures **34** and **36**). The contents of the stalk and lateral sac are continuous and consist of finely granular material regularly interspersed with coarser particles (figure **37**) which are best developed in the core of the shaft (figures **34** and **36**) and become sparse in the root region of the connecting structure (see for example, figure 42, plate 13). The shaft contains a system of poorly resolved, apparently single, fibrils (figure **34**) which extend the length of the shaft and are continuous with the root portion of the connecting structure (figure **38**, plate 12; figure 42).

Mitochondria dominate the cytoplasm of the long cone ellipsoid in l.a. and d.a. eyes

## Description of plate 7

Figure 22. Tangential section through the retina of a d.a. eye at a level between the outer and inner segments of the long cones. Cone 1 shows the ventral extension of the ellipsoid, with its mitochondria, and the dorsal position of the lamellae as the outer segment converges vitread on the connecting structure. Cones 2, 3 and 4 show further convergence of the outer segment towards the dorsotemporal side of the cone. Cone 5 is cut just vitread to the outer segment and obliquely through the connecting structure and base of the lateral sac. Note the sclerad extent of a few rod outer segments in the d.a. condition, and the temporal position of the lateral sac and connecting structure of the long cones. (Magn. × 10000.)

Figure 23. Longitudinal section through portion of the ellipsoids of a long cone (top) and a short cone (bottom) showing the difference between the mitochondria in the two cells and the general organization of the contact zone. (Magn. × 34000.)

## Description of plate 8

Figure 24. Vertical section through portion of the retina showing the outer segment of a long cone cut longitudinally and the stacks of reflecting platelets in the two adjacent pigment epithelium cells. The outer segment is wedge-shaped with a blunt asymmetric end joining the ellipsoid (bottom). Note the longitudinal orientation of the outer segment lamellae. The section is taken from a recently l.a. eye in which the melanosomes have not yet assumed their normal position at the level of the cone units; the cytoplasm is instead still dominated by tapetal crystallites. (Magn. × 13000.)

Figure 25. Vertical section through the sclerad end of a long cone outer segment showing the longitudinal orientation of lamellae in detail. The lamellae lie parallel to the ventral (right) face of the outer segment and meet the dorsal face (left) obliquely with closed ends. The cell membrane of the cone overarches the closed ends of the lamellae on the dorsal face and lies close beside the plasma membrane of the pigment epithelium cell forming a lamella-like structure (arrow). A similar structure occurs on the ventral face of the outer segment (double-barbed arrow) and lies parallel to the true lamellae. (Magn. × 35000.)

Figure 26. Transverse section through the vitread region of a long cone outer segment showing the horizontal orientation of lamellae and the temporal (left) position of the lateral sac. The lamellae have open ends on the nasal face (right) and open ones on the temporal. A large mitochondrion with tubular cristae abuts the outer segment at the sclerad end of the ellipsoid. (Magn. × 21000).

Figure 27. Transverse section of a d.a. eye through the ellipsoid of a long cone, at a level just sclerad to the lobes of the short cones of the cone unit. On either side of the long cone there is a space between it and the pigment epithelium, apparently caused by radial compression of the short cone lobes on dark adaptation of the eye. Note the paucity of mitochondria within the long cone ellipsoid at this level and the poorly developed expanded perimitochondrial spaces. (Magn. × 13500.)

(figure 14, plate 3; figure 17, plate 4; figure 22, plate 7; figure 34, plate 11; figure 48, plate 15). The distal region contains a group sandwiched between the outer segment and the pockets that enclose the lobes of the short cones. These mitochondria are orientated towards the connecting structure, except on the temporal flank (figure 34). On the ventral side of the cone they are smaller and closely packed. A similar condition occurs in the narrowed portion of the ellipsoid situated between the dorsal and ventral pockets (figure 48). By far the greatest concentration of mitochondria in the long cone occurs in the vitread region below the pockets (figure 48). In general, mitochondria are closely packed throughout the ellipsoid region, leaving little space for other organelles except towards the lateral margins of the cell. At the periphery of the ellipsoid they tend to be slightly smaller than those more centrally placed, a condition noted also by Dickson & Hollenberg (1971) in cones of the newt.

During photomechanical movements of the retina, the ellipsoid just sclerad to the pockets changes in length and this in turn affects the packing of mitochondria. In the l.a. state, the ellipsoid remains short and the mitochondria remain compacted. However, once the eye is fully dark adapted, and on its return to the l.a. state, this region of the ellipsoid elongates a

## Description of plate 9

Figure 28. Transverse section through the outer segment of a long cone and its associated stacks of guanine platelets lying within the two adjacent pigment epithelium cells. The lamellae of the outer segment lie perpendicular to the cone row (left to right), longitudinal to the axis of the cell and approximately horizontal with respect to the eye of the living fish. The lamellae are open along the nasal margin (top) and closed temporally (bottom). (Magn. × 28000.)

Figure 29. Transverse section of a cone unit at a level through the outer segment lobes of the short cones. The ellipsoid of the long cone is greatly narrowed in this region and partly encloses the lobes of the short cones. The lamellae of the short cones lie longitudinal to the cell and vertical in relation to the eye of the living fish. In both figure 28 and 29 the retina is not yet completely light adapted, as shown by some buckling of the cone lamellae and the occurrence of scattered rod outer segments at these levels in the photoreceptor layer. (Magn. × 25000.)

*Note.* For correct viewing of plate 9 relative to the axis in the living fish turn through 90°.

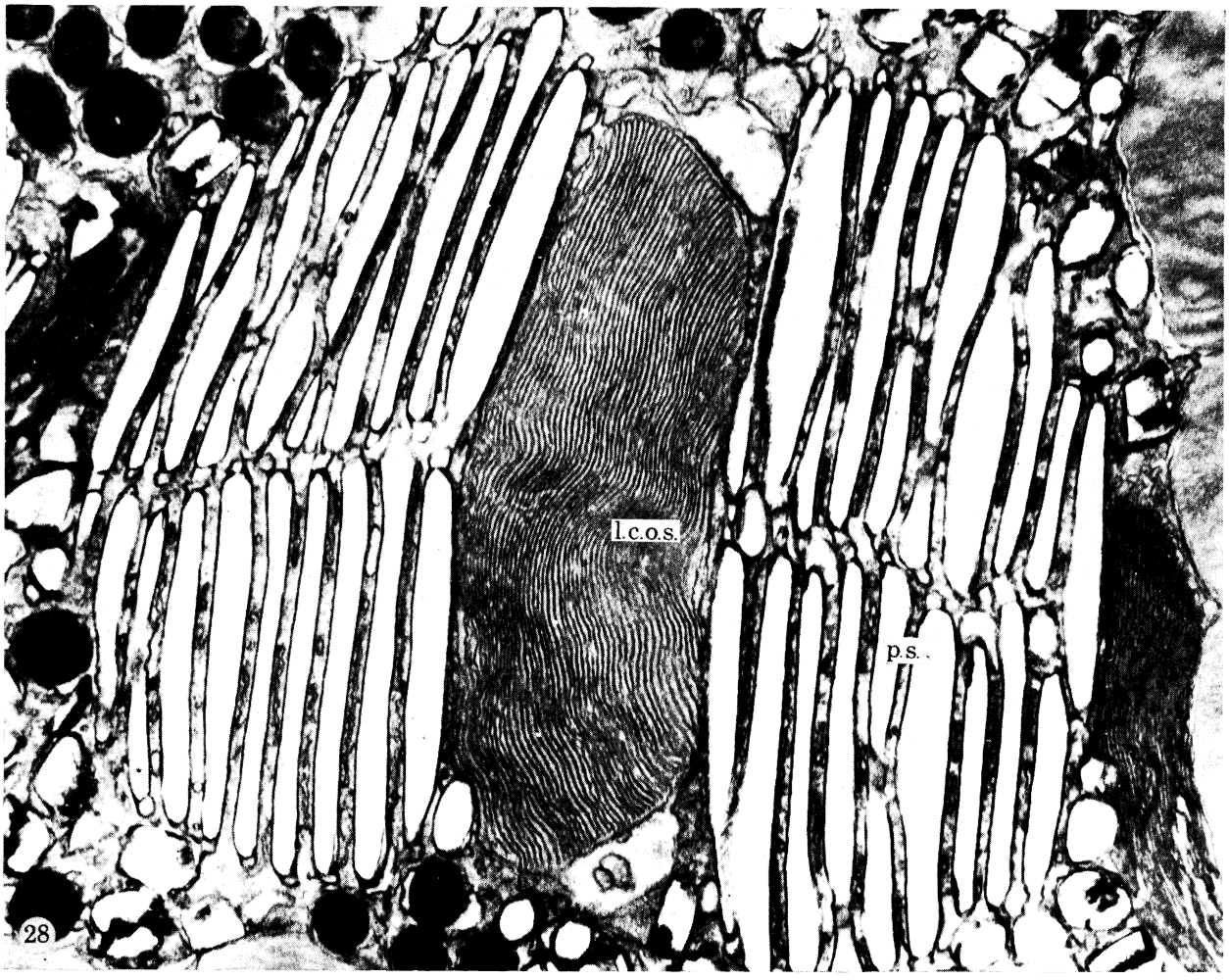
## Description of plate 10

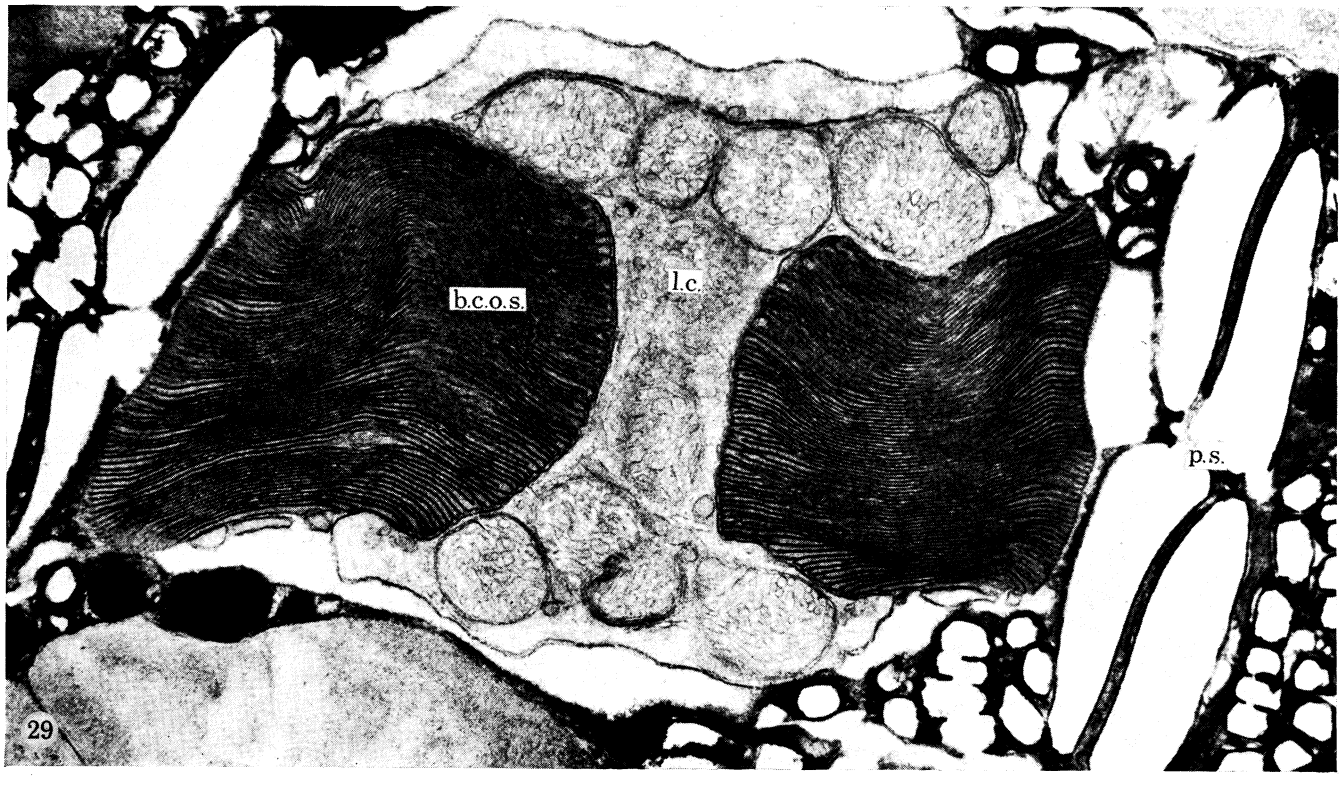
Figure 30. Cone units from the dorsal margin of the retina of a l.a. eye showing the orientation of lamellae in the long and short cones. In contrast to long cones elsewhere in the eye, those at the margins have transversely arranged lamellae. The lamellae of short cones retain their normal longitudinal orientation with respect to the axis of the cell. (Magn. × 14500.)

Figure 31. Long cones from the nasal margin of the retina showing the progressive change, from transverse to longitudinal, in the orientation of lamellae in successive cones away from the margin (left to right). Note the appearance of platelets (arrow) associated with those cones where the lamellae are becoming more vertical. In figures 30 and 31, note the essentially cylindrical shape of the outer segment. (Magn. × 9700.)

Figure 32. Tangential section through portion of the retina of a l.a. eye at and near the external limiting membrane. In the middle and top right the section has passed parallel to the external limiting membrane and shows the Müller cells surrounding rod inner segments with junctional complexes (arrows) developed between the two cell systems. On the left and bottom the section is slightly sclerad to the external limiting membrane and shows scattered rod myoids interspersed with transversely cut microvillus-like processes of the Müller cells. Well developed myoid folds are present on the nearby cones but are scarcely formed on the rods. (Magn. × 15500.)

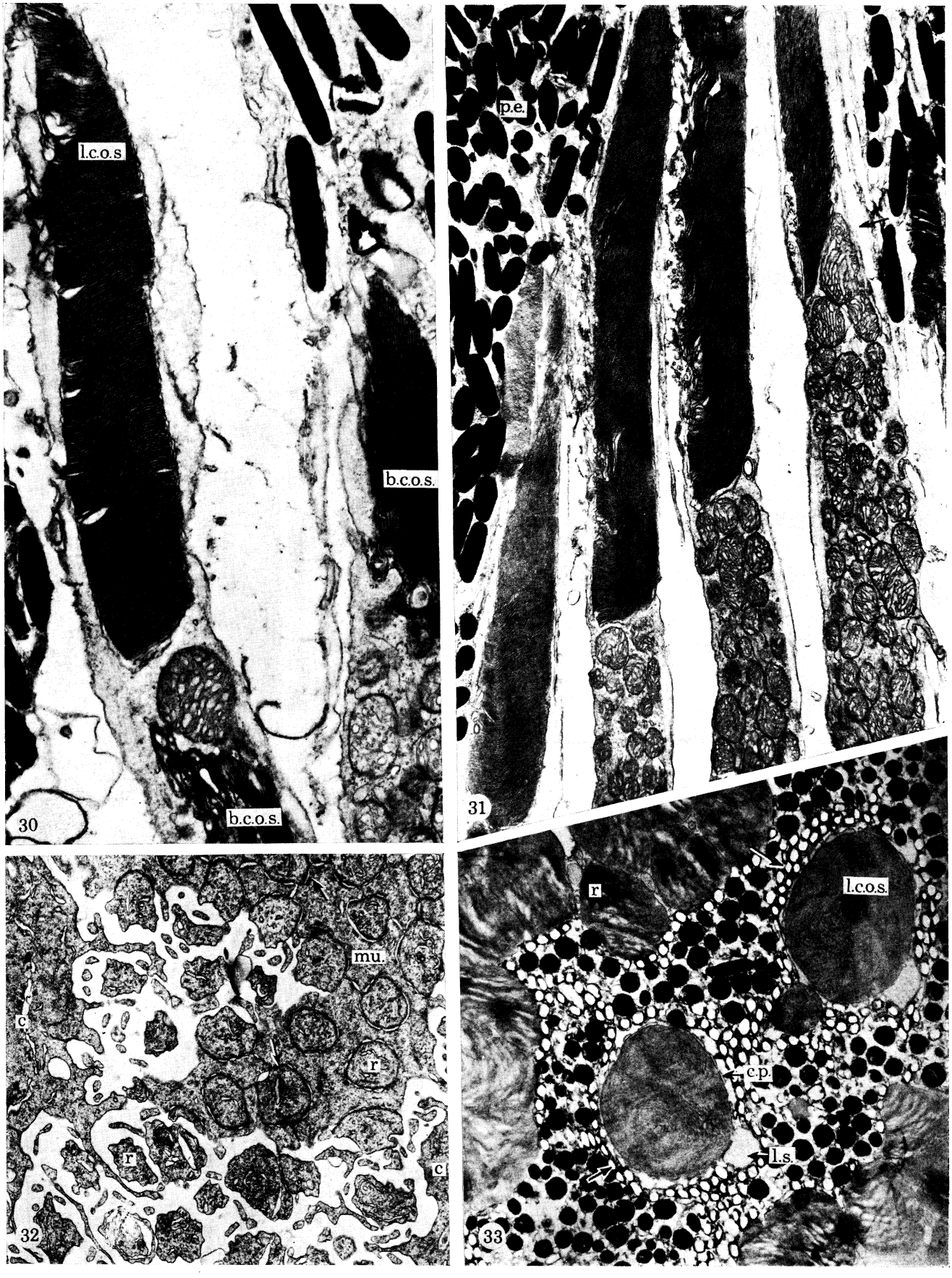
Figure 33. Tangential section through portion of the retina at its margin showing the outer segments of two long cones cut transversely. Unlike cones from elsewhere in the eye (see for example, figures 20 and 21, plate 6), these have a circular outline, are ringed by calycal processes, and have a 'finger print' pattern of cut lamellae. Within the associated pigment epithelium cells there is a ring of crystalline needles cut transversely (arrows) instead of the usual platelets, abutting the cones, and an abundance of melanosomes but few crystallites. (Magn. × 9000.)



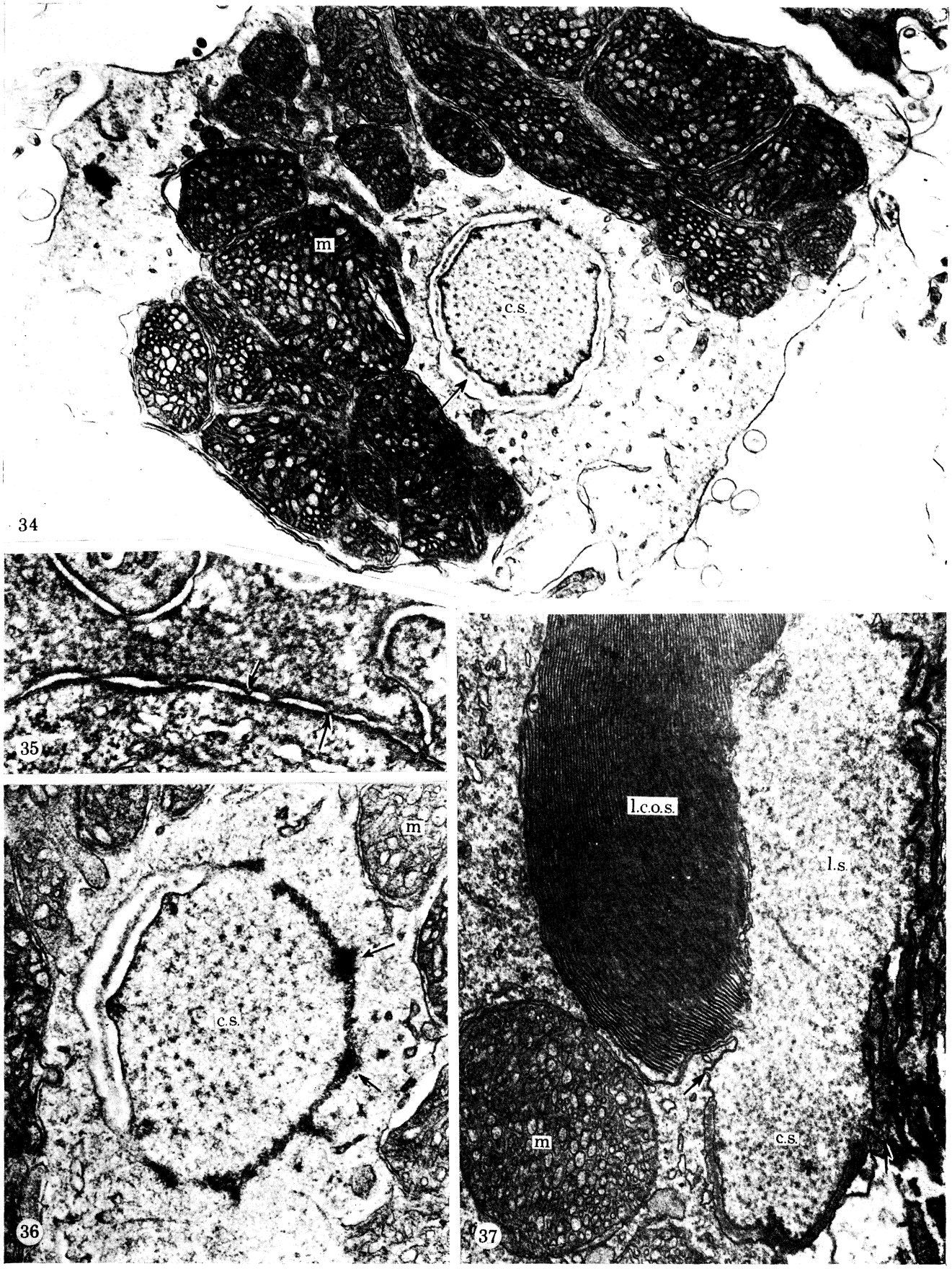




FIGURES 28–29. For description see opposite.

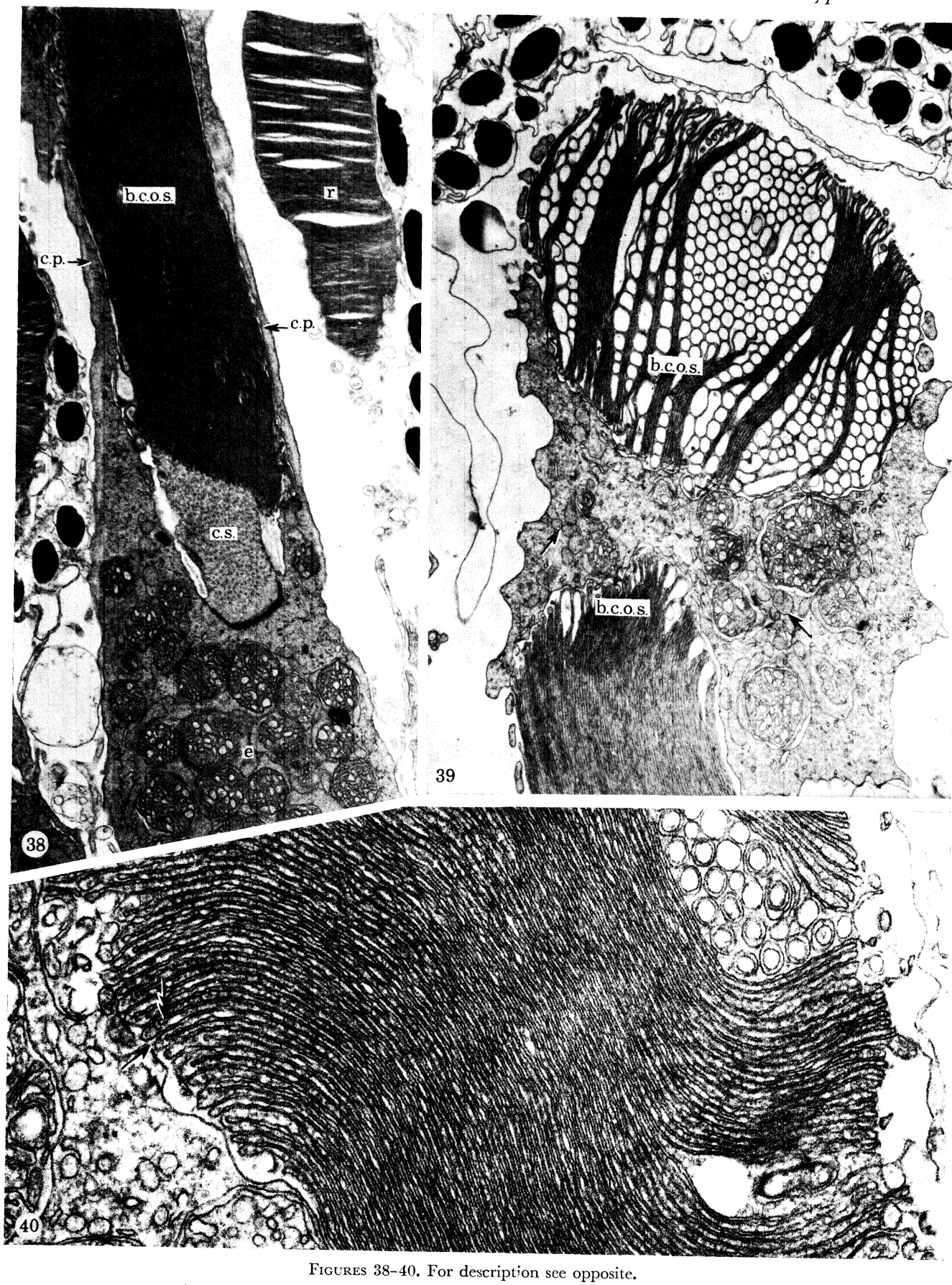
(*Facing p.* 38)




FIGURES 30–33. For description see page 38.




FIGURES 34–37. For description see page 39.




FIGURES 38–40. For description see opposite.

little and displaces the mitochondria. Spaces may also develop on either side of the cone between it and the adjacent pigment epithelium cells (figure 27, plate 8).

Mitochondria of the ellipsoid are mostly spherical or slightly elongated with the elongated ones orientated transversely to the axis of the cell and parallel to the lamellae of the long cone outer segment (figure 26, plate 8). They vary only slightly in size except adjacent to the myoid where they become noticeably smaller (figure 48).

An unusual feature is a perimitochondrial space which lies between the outer and inner membranes and is often greatly expanded to varying extents around the organelle (figure 23, plate 7; figure 43, plate 13). The condition is particularly well developed in l.a. eyes and is also noted in the d.a. retina to a somewhat lesser extent. The expanded perimitochondrial space of one mitochondrion is frequently compressed against that of adjacent mitochondria and the adjacent outer membranes superficially resemble profiles of endoplasmic reticulum. The

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## Description of plate 11

Figure 34. Transverse section of a long cone at a level through the connecting structure. Well differentiated closely packed mitochondria surround the connecting structure, except on its temporal side (right; see also figure 38, plate 12) where the cytoplasm contains mainly tubules and short cisternae of endoplasmic reticulum. The connecting structure has been cut through the stalk portion and shows an extracytoplasmic space (arrow) containing a ring of finely granular material. Within the stalk at its periphery there are spaced (irregularly so towards the bottom) nine small clumps of dense material. The ground substance of the stalk contains coarse and fine granular material. (Magn. × 22500.)

Figure 35. Tangential section through the external limiting membrane showing detail of the junctional complexes between the Müller cells and the photoreceptors. Note the zone of dark ground substance abutting the cell membranes and the points at which the opposing membranes approach each other (arrows). (Magn. × 25000.)

Figure 36. High power micrograph of a section obliquely cut through the stalk (left) and root (right) portions of the connecting structure. The corners of the root portion (arrows) are formed by dense material continuous with the fibrils of the stalk. Between the thickened corners are areas consisting of single rows of closely spaced fibrils. The fibrils superficially appear short due to the plane of sectioning. (Magn. × 33000.)

Figure 37. Longitudinal section through the vitread end of a long cone outer segment and its associated lateral sac and connecting structure. As the plane of section is lateral, only the stalk portion of the connecting structure is shown with its vitread expanded extracellular space and scleral (arrows) narrowed regions. On the left, the closely opposed cell membrane of the stalk is shown continuous with the lamellae of the outer segment. (Magn. × 23500.)

## Description of plate 12

Figure 38. Transverse horizontal section of the retina showing portion of the outer and inner segments of a short cone cut longitudinally. The section is slightly obliquely cut and passes through one lobe of the outer segment, the connecting structure and portion of the ellipsoid. The lamellae of the cone lie longitudinal to the axis of the cell in contrast to those of the nearby rod. Note in the l.a. eye shown here how the outer segment lobe is laterally compressed and radially elongated so that all the lamellae lie stacked flat against each other unlike those of the d.a. state (compare figure 20, plate 6). (Magn. × 13300.)

Figure 39. Transverse section of a cone unit of a l.a. eye through the outer segment lobes of the short cones and the enclosing ellipsoid region of the long cone. The lamellae of the lower short cone are mostly normal in appearance with a parallel arrangement, but in the upper lobe much of the lamellar system has become transformed into a system of closely packed tubules. The long cone ellipsoid contains mainly endoplasmic reticulum in the form of tubules and short cisternae, and mitochondria with expanded perimitochondrial spaces most of which appear separate from the organelle in this section (arrows). (Magn. × 19000.)

Figure 40. Transverse section through portion of an outer segment lobe of a short cone showing the interrelationship between lamellae and the way in which the lamellae are buckled in the d.a. condition. Where the lamellae abut the pigment epithelium cell (right) they have open ends with the cell membrane continuous from one lamella to the next. On the left the lamellae show both open (arrow) and closed ends with those having closed ends (double-barbed arrow) interdigitating with the open-ended ones which lie to the outside. (Magn. × 57000.)

material filling the perimitochondrial space is finely granular and has an electron density slightly greater than that exhibited by the adjacent ground substance of the cytoplasm.

The inner membrane of the mitochondrion is invaginated to form tubular cristae (figure 26, plate 8; figure 34, plate 11). The tubules are frequently swollen and are closely packed throughout the organelle; in the more elongated mitochondria they tend to be aligned lengthwise throughout the organelle. The stroma matrix of the mitochondrion is finely granular resembling that of the perimitochondrial space. A distinctive feature of many ellipsoid mitochondria is the presence of compact masses of membranous material resembling myelin configurations (figures 43 and 48), from the outer membrane of the organelle. The membranous mass either projects into the mitochondrion, usually the perimitochondrial space, or into the adjacent cytoplasm.

In addition to mitochondria, the ellipsoid contains other cytoplasmic components, mainly towards the periphery of the cell. Endoplasmic reticulum in the form of short cisternae and anastomosing tubules, mostly smooth, are common (figure 34). Tubular and fine fibrillar material extending longitudinally in peripheral parts of the inner segment (figure 50, plate 16), probably represent microtubules and microfilaments, respectively. The calycal processes contain ground substance and sometimes microfibrillar-like material.

A contact zone occurs along the dorsal and ventral faces of the vitread region of the long cone ellipsoid where it meets the ellipsoid of adjacent short cones (figure 45, plate 14; figure 48, plate 15). A single cisterna of smooth endoplasmic reticulum lies alongside the cell membrane of both cones and extends nearly to the lateral margins of the cell (figure 23, plate 7; figure 49, plate 16). The organization of this contact zone is similar to that described for double cones of other vertebrates (Engström 1963*a*; Berger 1967; Ahlbert 1973; Fineran & Nicol 1974).

The myoid of the l.a. long cone displays few mitochondria (figure 48) but scattered ones occur throughout the region in the elongated d.a. condition, particularly near the external limiting membrane (figures 15 and 16, plate 3). These mitochondria have narrow perimitochondrial spaces resembling those of typical mitochondria. The cytoplasm of the myoid contains mainly short profiles of endoplasmic reticulum that usually have a somewhat swollen appearance and clusters of ribosomes. The cell membrane is extended radially to form myoid folds which contain only ground substance material. These folds interdigitate with the microvillus-like processes of the Müller cells which in turn develop similar associations with the short cones, and rods, and other Müller cells (figure 32, plate 10; figure 35, plate 11).

(ii) *Short cones*

(1) *Outer segment.* The photosensitive lamellae of the short cone are arranged longitudinally in relation to the axis of the cell (figure 38, plate 12; figure 41, plate 13) with the lamellae of both lobes of the outer segment being continuous (figure 17, plate 4; figure 45, plate 14). The orientation of lamellae is, therefore, vertical with respect to the retina of the living fish, parallel to the cone row (figure 20, plate 6; figure 45) and at right angles to those of the long cone (compare figures 28 and 29, plate 9). Usually 100–120 lamellae make up an outer segment lobe, with the number of lamellae in adjacent lobes of the cone unit showing close agreement (figure 29). In l.a. eyes the lamellae are typically parallel (figures 17 and 38), but in the d.a. state they may become buckled (figure 20; figure 40, plate 12). The thickness of a lamella and inter-lamella space is the same as in the long cone outer segment. In both the l.a. and d.a. states the regular arrangement of lamellae is sometimes interrupted when they are transformed

into tubules (figures 39 and 40, plate 12) similar to those noted in the long cone outer segment. The tubules usually run longitudinally, with respect to the axis of the cell, but some groups run transversely. Most of the tubules contain electron transparent extracellular material and their limiting membrane appears to have assumed its tubular configuration as a result of local fusion between the opposite membranes of a lamella. Occasionally double membrane-bounded tubules occur (figure 40).

As in the long cone, the lamellae of a short cone are formed from a folded single membrane system which in the mature photoreceptor is continuous throughout the stack and with the plasma membrane. The lamellae along the inner face of each lobe facing the pigment epithelium cell have open ends (figure 29, plate 9: figure 44, plate 13) but those within the stalk region have closed ends (figure 41, plate 13). However, along the outer dorsal and ventral faces of the lobes, beyond the insertion into the connecting structure, the situation is complex with open and closed lamellae occurring together (figure 28, plate 9). Typically, on these faces the closed lamellae predominate and the open ones alternate or are spaced randomly at greater intervals (figure 40).

Marginal vesicles are present along the outer segment lobes (figure 44). They are numerous on the inner dorsal and ventral faces and are associated with the open ends of the lamellae. In some cones there is also a small differentiation of marginal vesicles on the outer dorsal and ventral faces, but their occurrence here is associated only with those sets of lamellae having open ends.

(2) *Inner segment.* The connecting structure of the short cone resembles that of the long cone in basic organization. It is situated towards the nasal face of the ellipsoid and is slightly oblique to the longitudinal axis of the cell (figure 41). The lamellae of the outer segment exhibit their greatest vitread development towards the nasal side of the stalk (figure 42, plate 13). Typically, in l.a. eyes, the inner region of the stalk is cylindrical, but immediately beneath the outer segment lamellae it widens and resembles a slightly flattened truncate cone (figure 45, plate 14). In d.a. eyes the sclerad region of the stalk usually becomes elongated (figures 46 and 47, plate 14).

Each inner segment usually has between five to seven calycal processes extending sclerad along each lateral face of the outer segment lobes (figures 29 and 39). The processes are slightly flattened laterally at their proximal end and taper to a fine point distally.

The ellipsoid is filled with well differentiated mitochondria (figure 38, plate 12; figure 41, plate 13; figure 46, plate 14; figure 48, plate 15). Mitochondria resemble those of the long cone in their development of tubular cristae, an expanded perimitochondrial space and associated myelin-like membrane configurations; the tubular cristae are a little narrower and are more highly concentrated within the organelle (figure 23, plate 7). Throughout the ellipsoid, mitochondria lie close to the dorsal and ventral faces of the cell (figure 23). On the lateral faces of the ellipsoid there is a narrow zone of cytoplasm that contains tubular and short cisternoid endoplasmic reticulum, scattered clusters of ribosomes and some microtubular and fibrillar material amongst the otherwise structurally poorly differentiated ground substance (figure 41).

The myoid region of the short cone is very short in the l.a. eye and is half the length of the myoid of the adjacent long cones (figure 18; figure 48). Myoid folds are well developed on the lateral faces of the cell and contain mainly ground substance; they intermesh with the microvilli-like processes of the Müller cells (figure 15, plate 3).

There is a well differentiated contact zone on the horizontal faces of the ellipsoid (figure 23;

figure 45, plate 14). The zone is characterized by the occurrence of a smooth sheet of subsurface cisternoid endoplasmic reticulum. The cisterna is mostly entire throughout its extent; nevertheless, scattered fenestrations do occur within it in both cones (figure 49, plate 16). Button-like adherent areas are also present between the adjacent cell membranes of the cones (figure 49). These adherent points are usually more numerous towards the margins of the contact zone where it lies close to the space between the rows of cones. In the myoid region the cell membranes of the two cones remain in proximity but the subsurface cisternae are poorly developed, being largely replaced by a diffuse system of apparently tubular endoplasmic reticulum. Adherent points between the cell membranes of the two cones occur in the myoid but appear to be less numerous than in the ellipsoid region.

(iii) *Rods*

The ultrastructure of the rods in *Anchoa* resembles that found in the eyes of other vertebrates. The outer segment photosensitive membranes form closed lamellae (figure 21*b*, plate 6), or disks, arranged transversely to the axis of the cell (figure 38, plate 12). Very occasionally, a small group of the outermost lamellae is found to be folded and lying partly longitudinal to

## Description of plate 13

Figure 41. Longitudinal section through a short cone showing the region between the outer and inner segments. The section is horizontal, with respect to the retina in the living fish, and therefore shows the outer segment lamellae in profile and the connecting structure lying more towards the nasal (right) side of the cell. The ellipsoid contains abundant well differentiated mitochondria. (Magn. × 22500.)

Figure 42. Detail of the connecting structure of another short cone in longitudinal section. The ground substance of the stalk portion contains coarse and fine granular material whereas that of the root has only a finely granular appearance. Portions of the fibrils of the stalk are shown in section (arrows) and at one point (double-barbed arrow) continuous with the root portion of the connecting structure. (Magn. × 17000.

Figure 43. High magnification of a group of mitochondria from the ellipsoid of a short cone showing the closely packed tubular cristae, dark myelin-like membrane configurations and expanded perimitochondrial spaces (arrows) filling the regions between these mainly spherical shaped mitochondria. (Magn. × 25000.)

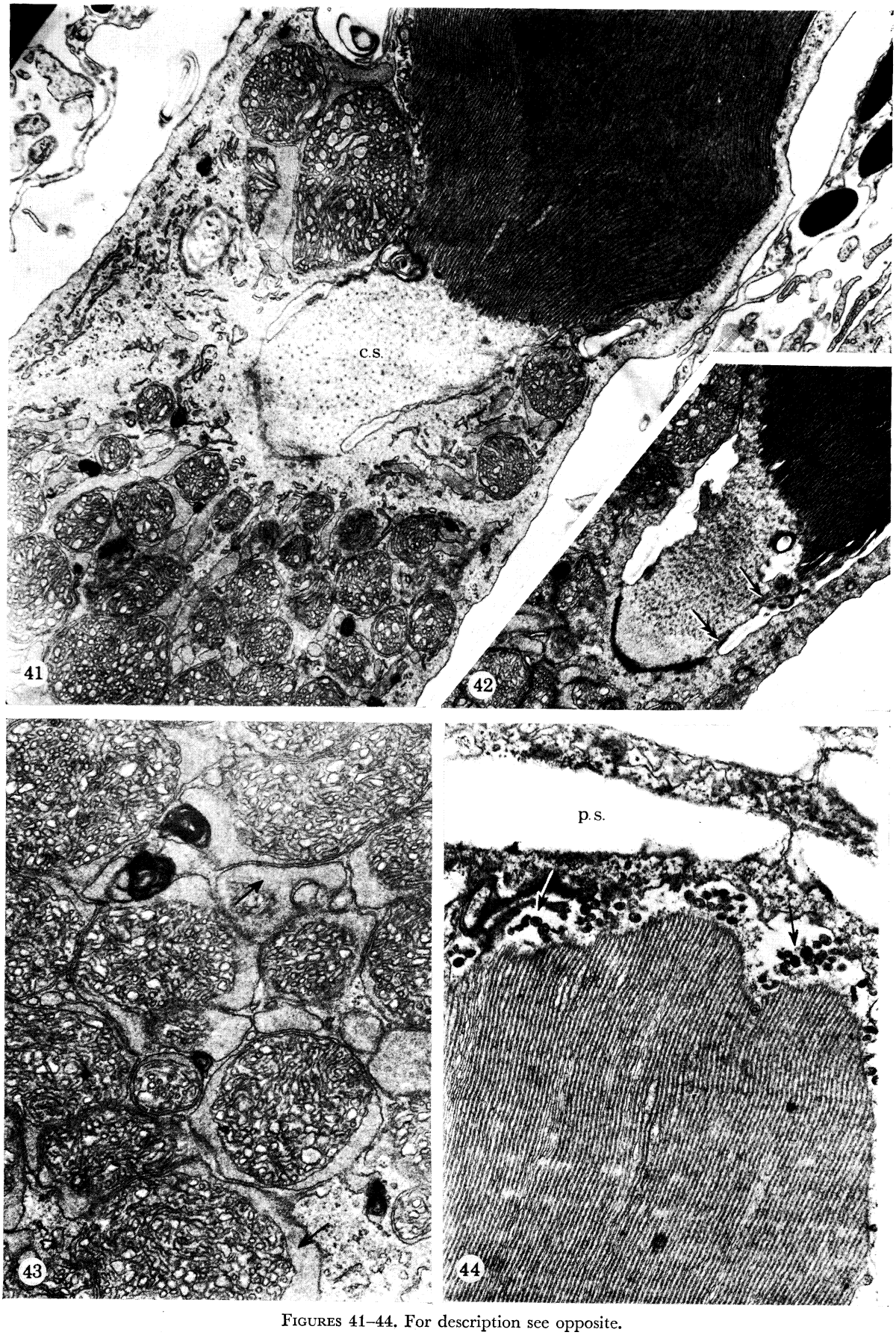
Figure 44. Transverse section through the outer margin of a cone lobe where it faces the platelet stacks of the adjacent pigment epithelium cell (upper). The lamellae along this margin of the outer segment have open ends and the membrane is continuous between lamellae. Between the lamellar stack and the pigment epithelium cell is a zone containing a system of peripheral vesicles (arrows). The contents of these structures are dense compared to the ground substance between the lamellae, with which they are continuous, and some also show an internal core. (Magn. × 28000.)

## Description of plate 14

Figure 45. Transverse section through portion of a cone row at the vitread end of the short cone outer segment showing the alternate sequence of long and short cones and the short cone lamellae aligned with the vertical orientation of the cone row. The short cone on the right shows part of the underlying connecting structure while the cone on the left shows the lamellae continuous from one lobe to the other at a level just vitread to the notch between the lobes. The buckling of lamellae in the left cone and the occurrence of some rod outer segments bordering the cone rows suggests that this eye is not fully light adapted. Note the well developed contact zone between the cones in this and the next figure. (Magn. × 15700.)

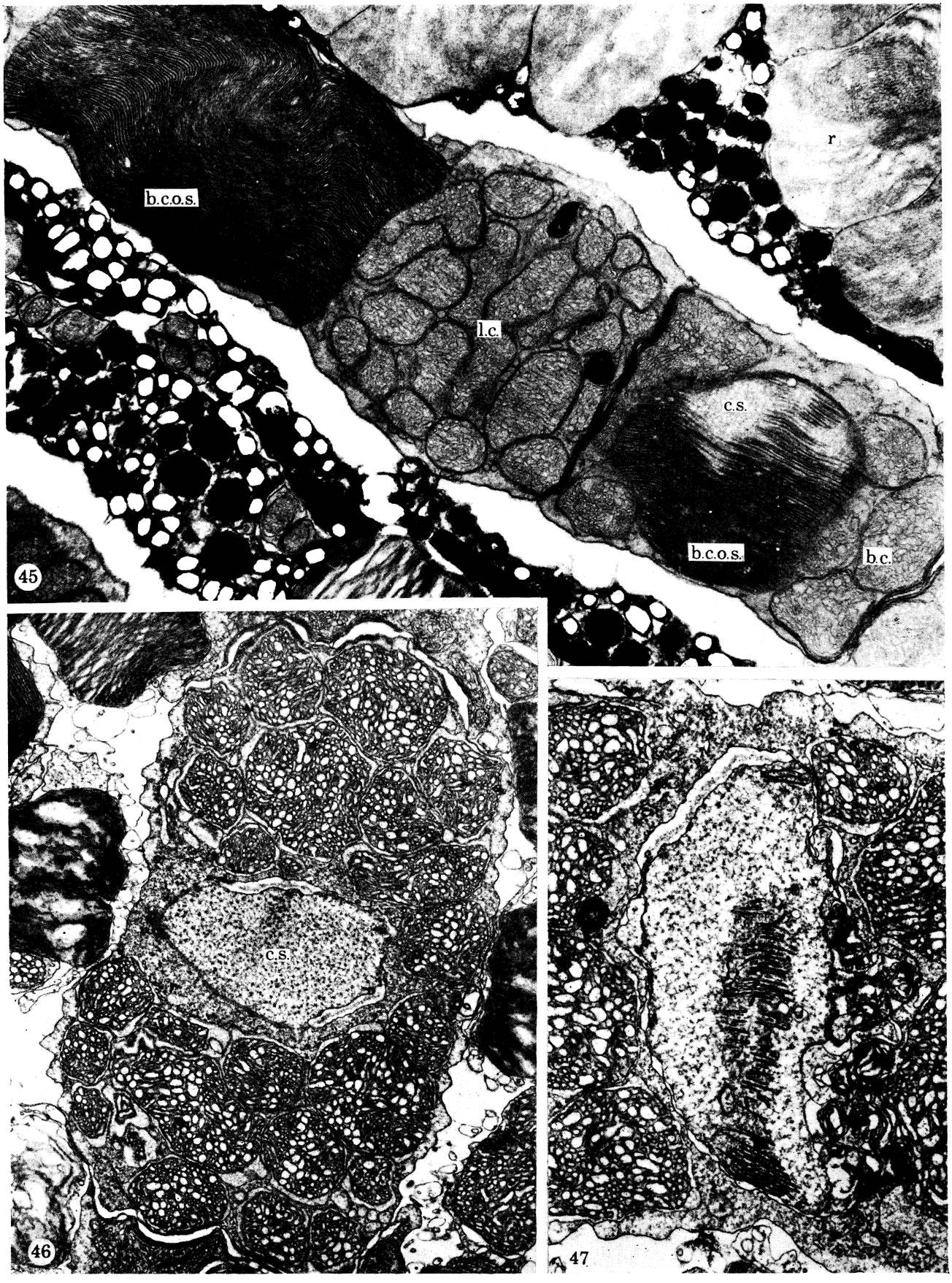
Figure 46. Transverse section through the ellipsoid of a short cone from a d.a. eye showing the dense packing of mitochondria and the connecting structure cut at slightly different levels through the stalk and root portions. Note the position of the connecting structure more towards the nasal (left) side of the cone and its vertically compressed oval shape in the d.a. condition. (Magn. × 15300.)

Figure 47. Detail of the connecting structure and vitread portion of the outer segment lamellae of a short cone from a d.a. eye. The expanded extra-cellular space of the stalk is shown at top left, but elsewhere the section passes more sclerally and the cell membrane is closely apposed. (Magn. × 24500.)



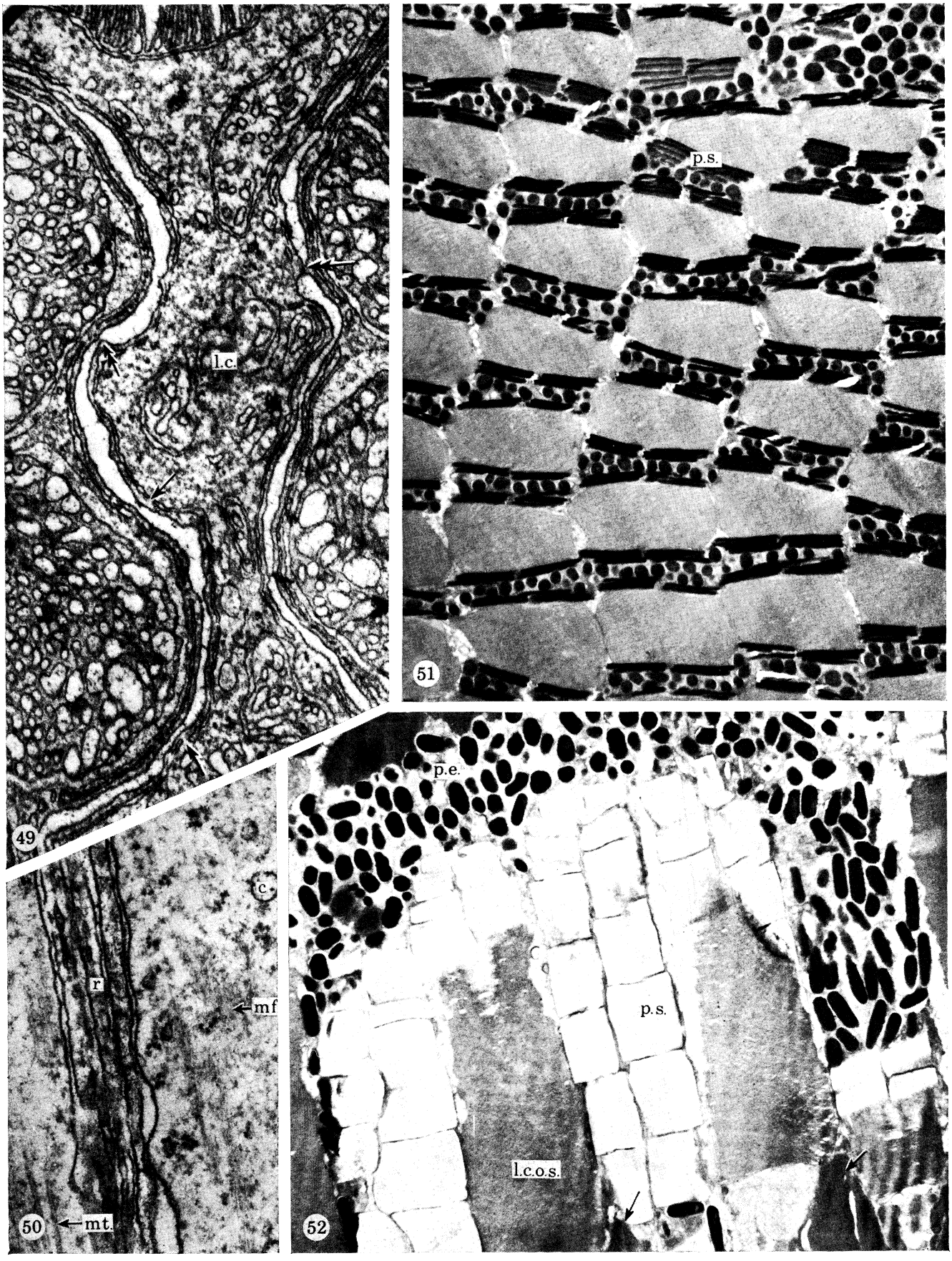

FIGURES 41–44. For description see opposite.

(*Facing p.* 42)




FIGURES 45–47. For description see page 42.


FIGURE 48. For description see page 43.




FIGURES 49–52. For description see opposite.

the axis of the cell in a manner resembling that described by Locket (1971 *a*) in rods of the deep sea fish *Platytroctes*. In well fixed specimens of *Anchoa* the diameter of a rod disk is greater than that of a cone lamella (approximately 1.5:1) but the inter-disk/lamella space in both photoreceptors is the same (figure 21). However, frequently the intra-disk space is variable in thickness as a result of swelling during specimen preparation (figure 38). The stack of discs is often penetrated by a longitudinal incision or cleft that sometimes forks, forming short branches within the stack. The incision penetrates the stack to about a third its diameter. The outer and inner segments are connected by a short cilium process situated to one side of the ellipsoid and lying within a pocket; the cilium shows the usual construction of 9 + 0 axial filaments anchored to a centriolar or basal body structure. The rod ellipsoid is short and filled with a small number of mitochondria which are often elongated in the direction of the cell and contain a well developed tubular system of cristae. In the l.a. eye the rod myoid is very narrow and contains mainly ground substance, ribosomes, and endoplasmic reticulum that is usually tubular or in profiles of short cisternae. Myoid folds are poorly differentiated.

## Description of plate 15

Figure 48. Portion of a l.a. eye showing the vitread region of a cone row cut longitudinally and the alternating sequence of long and short cones. In the micrograph a short cone is visible, just right of centre, flanked by the inner segment regions of two long cones. Between the outer segment lobes of the short cone lies the vitread process of a pigment epithelium cell with two stacks of reflecting platelets that meet at the very tip of the cell. At this level, along the length of the short cone outer segment, there are only a few rows of platelets within each stack in contrast to the situation at the sclerad end of the cone unit (see figure 24, plate 8). Large well differentiated mitochondria dominate the cytoplasm of the ellipsoid of both cones, except in the myoid region. The myoid of the short cone is shorter than that of the long cone. A contact zone, characterized by sub-surface cisternae, extends the length of the adjoining ellipsoids of the long and short cones but is not developed in the myoid regions. (Magn. × 12 600.)

## Description of plate 16

Figure 49. Detail of the contact zones between a long cone (centre) and its adjacent short cones in a d.a. eye. Each contact zone is characterized by the presence of a single sheet of smooth cisternae developed close to the plasma membrane of each cell. Although many of the cisternae appear to be entire, close examination reveals small fenestrations (arrows) scattered throughout each sheet. At various points throughout the zones, especially towards the margins, the opposite cell membranes approach each other forming what appear to be points of adhesion (double-barbed arrows). (Magn. × 50 000.)

Figure 50. High power detail of portion of the inner segments of two cones separated by a bundle of rod myoids (left centre). Structures resembling microtubules and bundles of microfilaments are resolvable amongst the scattered clumps of ribosomes and the otherwise structurally poorly differentiated ground substance of the cell. (Magn. × 40 000.)

Figure 51. Tangential section through the retina of a larval eye of *A. mitchilli* showing vertical rows of long cones of the cone units cut at slightly different levels towards the tips of the cells (top). There is little change in the shape of the cells sclerally indicating that the outer segment of the long cone is a laterally flattened cylinder with little taper. At this stage in the development of the eye the stacks of platelets (represented by dark bars) of the associated pigment epithelium cells are poorly developed and consist of only a few rows; the remaining cytoplasm of the cell is dominated by melanosomes. The 'finger print' pattern of the lamellae of the long cones indicates that they are mostly transversely arranged. Electron micrograph through the courtesy of Dr H. J. Arnott. (Magn. × 7200.)

Figure 52. Transverse horizontal section through the cone layer in a larval eye of *A. mitchilli* showing the outer segments of some long cones and the sclerad tips of two short cone outer segment lobes (arrows). In contrast to the condition in the adult retina (figure 24, plate 8), the lamellae of the long cone are transversely arranged and therefore resemble those in a normal vertebrate cone. Although barely visible in this micrograph, the lamellae of the short cones are vertical as in the eye of the adult fish. Electron micrograph through the courtesy of Dr H. J. Arnott. (Magn. × 7200.)

(iv) *Interrelation between photoreceptors and Müller cells at the external limiting membrane*

At the external limiting membrane the rods and cones are separated by Müller cells (figure 32, plate 10). Rod myoids become completely enveloped by portions of Müller cells, and other processes of the Müller cells extend between the long and short cones. Where Müller cells meet the rods and cones at the level of the external limiting membrane, junctional complexes or regions of intercellular adhesion are developed but we have not attempted to study these in detail. Throughout the length of the zone there is a layer of dense material lying on the cytoplasmic side of each membrane. The appearance of these junctional zones is shown in plate 11, figure 35.

### (*e*) *Differentiation of the cones*

The differentiation of the cones was examined briefly in *A. mitchilli* to determine their relation to those of normal cones. Since during growth of the retina differentiation of photoreceptors takes place last at the margins (Coulombre 1965), sections were cut through the margins of adult eyes. A few observations were also made on cones in the eyes of young fish, based on a small collection of electron micrographs taken by Dr H. J. Arnott during studies on the tapetum (Nicol *et al.* 1973).

In the most marginal cones the outer segment is cylindrical or steeply conical and somewhat truncate surrounded by a ring of calycal processes (figures 30, 31 and 33, plate 10). In the course of differentiation the outer segment of the long cone is transformed into a cuneate structure. Initially, it seems, there is local growth on the dorsal and ventral sides of the outer segment leading to a change from a cylinder to a form that is somewhat laterally compressed. As a consequence of this growth, the calycal processes are separated into two groups: one set lies on the temporal side of the outer segment and is divided by the lateral sac, the other set comes to lie alongside the nasal margin. In addition to the flattening of the dorsal and ventral faces of the outer segment, there appears to be concommitant growth, particularly on the dorsal side, transforming the outer truncate end of the cone into a narrow edge. These changes in the morphology of the outer segment from a cylinder to a wedge are also accompanied by internal changes of which the most important is a shift in the alignment of the lamellae from transverse to longitudinal.

Various stages in the transition of the outer segment of the long cone from the juvenile to the adult condition may also be interpreted in sections from the retina of young fish. In the least developed eyes available to us the long cones have cylindrical outer segments; in eyes of somewhat older fish the outer segment shows some lateral flattening and radial tapering (figure 51, plate 16). However, in most of the eyes of these young fish the lamellae of the outer segment remain chiefly transverse (figure 52, plate 16). In the larval herring (*Clupea harengus*) Blaxter & Jones (1967) found that the retina was composed only of cones and there was no retinomotor activity. As far as the electron micrographs available to us show, the larval eye of *A. mitchilli* is dominated by cones and rods also appear to be absent.

The differentiation of the short cone is not as well understood as that of the long cone. Sections through the margins of adult eyes reveal short cones with bilobed outer segments already formed and with longitudinally orientated lamellae (figure 30), although these may be fewer in number than in cones from positions lying more centrally in the retina. Similarly, in the eyes of young fish examined the short cones are seen to be fully differentiated, even in those

retinae where the outer segment of the long cone is cylindrical with transversely orientated lamellae (figure 52).

The way in which the long cone and the short cones come together during development to form the cone unit has not been studied closely. It seems that the long and short cones initially arise independently and as they assume positions further removed from the margin, on subsequent growth of the retina, they become associated in a vertical series through the establishment of contact zones on their dorsal and ventral faces.

The appearance of cone units in adult eyes is intimately associated with a change in the structure of the adjacent pigment epithelium, in particular with the formation of stacks of guanine platelets in the vitread region of the cell. In *Anchoa* stacks of platelets are a feature of the cone unit system throughout the retina (Fineran & Nicol 1977), except for the most marginal rows of cones where the cuneate cone unit is not fully differentiated. Only with the acquisition of its characteristic cuneate shape, in cone units progessively removed from the margins of the retina, is there a change in the structure of the pigment epithelium from a condition where the cells contain a system of needles surrounding the cylindrical outer segment of the cone (figure 33) to one dominated by stacks of platelets facing the dorsal and ventral sides of the cone unit. In the eyes of young fish changes are also noted in the organization of the pigment epithelium associated with the differentiation of the cones. At the stage where the long cones have cylindrical outer segments and transverse lamellae the pigment epithelium contains only a few rows of platelets. In somewhat older eyes as the outer segment becomes progressively cuneate, accompanied by a change in the orientation of lamellae from transverse to longitudinal, there is an increase in the number of rows of platelets.

### (*f*) *Polarization of reflected light*

Light-adapted eyes of anchovies were opened by removing the cornea and lens. The fundus was viewed under a dissecting microscope at magnification $\times 50$. It was illuminated from above at an angle of about 22° and a polarizer was rotated about the eyepiece. As the preparation or the polarizer was rotated, the retinal surface became alternately light and dark at each rotation of 90°. It was bright (green) when the plane of vibration ($\sigma$-component) was perpendicular to the direction of the vertical rows, and dark when the plane of vibration was parallel to the vertical rows. A piece of shiny aluminium foil, under water, was examined in the same manner. No perceptible diminution of intensity was detected by eye on rotating the polarizer through 90°.

## Discussion

In comparison with the eye of a typical vertebrate, the structure and arrangement of the cones of the anchovies *Anchoa mitchilli* and *A. hepsetus* are unusual. The extent to which the features described here pertain only to these fishes is not known, but studies in progress on two other genera of the Engraulidae indicate that at least some of the structures occur in other members of the family.

### (*a*) *Mosaic of photoreceptors*

A fundamental feature in the organization of the retina in *A. mitchilli* and *A. hepsetus* is the grouping of photoreceptors into rows that run vertically throughout the retina. This alternating arrangement also occurs in the pigment epithelium, whose cells form rows along the same radii

as those of the cones, and the masses of rod outer segments. The distribution pattern of the photoreceptors is determined by the grouping of the cones; the rows of rods are regarded as being a consequence of this arrangement. Whereas the cones show a very regular relation in the way they form rows, rods exhibit no specific association with each other in the multiseriate partitions in which they occur.

This arrangement of cones in *A. mitchilli* and *A. hepsetus* is a type of cone mosaic, but one that is different from the mosaics usually found in eyes having a regular distribution of photoreceptors. Engström (1963*a*, *b*) concluded that there are two fundamental types of arrangement of cone mosaic in teleost retinae: (*a*) rows of parallel arranged double cones sometimes alternating with rows of single cones and (*b*) patterns of squares. He considers that the square pattern develops from the row pattern and is evolutionarily more advanced. The most important differences between the row mosaics of other teleosts and those of *Anchoa* is that the cone rows of the latter form continuous uniseriate partitions, with all the cones being linked by contact zones similar to those developed between the members of a double cone. Such a mosaic of polycone complexes must be unique for a vertebrate eye, as far as is recorded in the literature. In the only other structural study of an eye from the Engraulidae, O'Connell (1963) gives information on the density of cones in *Engraulis mordax* and *Anchoa compressa* but he does not mention whether the cones are arranged in rows. In re-examining the eyes of *E. mordax*, with particular reference to its ultrastructural organization, we have found (unpublished observations) that cone rows do exist in this species, at least in certain parts of the retina. Similar exploratory work on the eye of *Stolephorus*, an anchovy from Indonesia, also indicates that cone rows occur in a third member of the family. In *Anchoa* the grouping of the cones into continuous rows is undoubtedly related to the structure and type of cone cells present and the way in which they might function together with the tapetum of the pigment epithelium in the perception of light.

In contrast to our observations on the Engraulidae, linear rows of cones in other teleosts do not form a continuum of structurally interlinked cells, at least sclerad to the external limiting membrane. In almost all known cases of teleosts exhibiting cone mosaics the pattern is made up of double cones sometimes incorporating single cones. In a table summarizing cone patterns in teleosts, Lyall (1957) shows a parallel arrangement of alternating double cones in *Gadus*. In studying the development of cone patterns in several fishes Lyall did, however, find a parallel arrangement at the growing margins of the retina and she came to the conclusion that 'a parallel arrangement of double cones is probably the basic pattern from which all others can be derived by positional changes and the addition of single cones' (Lyall, 1957). Rows of double cones have been described in many species (Bathelt 1970; Wagner 1972; Borwein & Hollenberg 1973). A change from rows to a square pattern was noted by Ahlbert (1973) in eyes of perch fry; the square pattern developed later during the ontogeny of the eye with the formation of double cones. These double cones arise through the opposition of two single cones which subsequently develop a contact zone along their adjacent faces. From observations based on tangential sections through the margins of the retina in *A. mitchilli*, parallel rows of cones are also developed initially, and as in the perch fry, these are made up of single cones. The main difference between *A. mitchilli* and other fishes which develop cone mosaics is that the long and short cones in a row series become structurally linked to form polycone complexes rather than forming rows of double cones which subsequently differentiate into patterns of squares.

The cone mosaic in *Anchoa* has a geometrical arrangement and cone units are alternately

placed between rows. The precision of the mosaic applies not only to the arrangement of the long and short cone cells but also to various of their cell components. Throughout the cone mosaic the lateral sac of the long cone, the orientation of long cone outer segment lamellae and the location of the connecting structure have the same relative position to that of other long cones. The location of the connecting structure of the short cone is also the same throughout the retina. A consistent position for the connecting cilium is not unknown among other vertebrate eyes which have a regular arrangement of cones (see for example, Locket 1971*b*). The precise geometry of the cone mosaic within the eyes of these anchovies probably results from a highly ordered differentiation of the cones during the ontogeny of the retina.

### (*b*) *Morphological relation between the long and short cones*

The cones are closely held together by contact zones on the dorsal and ventral sides of the cells and this leads to the formation of the polycone complexes that extend vertically throughout the eye. Intimate associations between cone cells, with the differentiation of contact zones, are known to occur in retinae of other vertebrates, especially those exhibiting double cones (Engström 1963*a*; Berger 1967; Ahlbert 1973; Fineran & Nicol 1974). The linear rows of cones in anchovies may represent a structural and functional continuum with each row of cells functioning as an integrated entity coordinated with that of adjacent rows. The presence of a close structural association between the outer regions of the cones and the processes of the pigment epithelium cells gives this idea further support, especially as the relation is maintained during photomechanical movements of the retina.

The polycone complexes of *Anchoa* are constructed from long cones and short cones. The inner segments are closely linked and the outer segments are also intimately associated so that much of the light received by the outer segment of one cone follows a similar path to that of light entering the other cone. The situation is further complicated by the fact that the short cone outer segment is in the form of two lobes, with one lobe associated with a long cone on one side of the cell and the other lobe with the long cone on the opposite side. The short cones therefore form a structural, and undoubtedly, functional link between the long cones and cone units of a cone row. As the lobes of the short cones lie partly within pockets of the long cone ellipsoid, this means that the lobes of the short cone outer segment lie almost directly beneath the outer segment of the long cone. Therefore much of the light received by the long cone outer segment will first pass through the outer segment lobes of the short cones.

Except at the margins of the eye, the photoreceptor cone layer in adult *Anchoa* is composed entirely of cone units with their characteristic cuneate morphology. Cone units displaying the same basic type of organization have now also been found by us in the eyes of *Engraulis mordax* and *Stolephorus*. The short cones of *E. mordax* appear less well developed than those of *A. mitchilli* and *A. hepsetus* in terms of the relative size of the outer segment and the extent to which it is enclosed by the ellipsoid of the long cone. Furthermore, in *E. mordax* the cone layer of retina does not appear to be very homogeneous but to consist of some areas with double cones, some with double cones intermixed with single cones and others of pure cone units. In *Stolephorus* the short cones so far observed are well developed and resemble closely those of *Anchoa*.

### (*c*) *Morphology and differentiation of the cones*

The regular bilobed character of the outer segment of the short cone is a most unusual condition. However, how the short cone differentiates into an outer segment with two lobes

containing vertically arranged lamellae remains to be investigated. With the exception of our observations on the Engraulidae, no other instances have been recorded where the outer segment of a cone is divided, apart perhaps for some cones of the pigeon (Cohen 1963). Cohen obtained evidence for the existence of cone varieties where the outer segments had one, two or three independent columns of lamellae, but he could not correlate these features with single or double cone varieties based on inner segment characteristics. It is of interest to note that the short cone only makes its appearance in those eyes, or parts of the retina, where the cones are arranged into polycone complexes. For example, in *Engraulis mordax* short cones seem to be absent in those parts of the retina containing double cones and appear only in those regions of the eye where the cones form linear polycone complexes.

The shape of the outer segment of the long cone is unusual. The cuneate form of the long cone is developed only on the formation of the cone unit and such a shape is absent in cones at the margin of the retina (figures 30, 31 and 33, plate 10). The long cone is thus a highly specialized cell derived during development from a cone of a more basic structure. The differentiation of the outer segment of the long cone is also intimately coordinated with the development of the pigment epithelium and we suggest that the acquisition of regular stacks of platelets is related to the functioning of an outer segment with essentially longitudinally orientated lamellae. In *Engraulis mordax* we have also noted that the stacks of platelets appear only in association with the formation of cone units and are absent alongside the double cones.

### (*d*) *Ultrastructural organization of photoreceptors*

#### (i) *Outer segment*

The most distinctive feature in the internal organization of the long cone and the short cones is the regular orientation and arrangement of the photosensitive lamellae of the outer segments. In both cones the lamellae are longitudinal, parallel to the axis of the cell. Apart from the two other members of the Engraulidae that we are studying (*Engraulis*, *Stolephorus*), where longitudinally orientated lamellae have been found in the short cone, and to some extent in the long cone, the condition in the vertebrate cone is for the lamellae to be orientated perpendicular to the axis of the cell. However, there are a few instances reported where abnormal arrangements of lamellae may occur in photoreceptors of otherwise normal retinae. Pedler & Tansley (1963) comment on abnormal arrangements in cones of a diurnal gecko and illustrate portion of one cone where some of the lamellae appear to have a longitudinal orientation. Earlier, Yamada and his colleagues (Tokuyasu & Yamada 1959) found in the cat and man rods having some irregular lamellae which they considered might even lie longitudinal to the axis of the outer segment.

The longitudinal orientation of lamellae in *Anchoa* is, without doubt, a highly modified and specialized condition derived from a situation where the lamellae are initially transverse. In the case of the long cone the development of longitudinally orientated lamellae can be followed ontogenetically in cones at the margins of the retina. This is in contrast to the condition so far noted in short cones where the lamellae are longitudinal from their inception in cells at the margin of the eye. The few observations that we have made on the eyes of young *A. mitchilli* show short cones with longitudinal lamellae at a stage when many of the long cones retain a transverse arrangement. Exploratory studies on the eye of *Engraulis mordax* also show short cones with longitudinal lamellae in a cone system where the long cones appear to have predominantly transversely arranged lamellae, or at most have only small areas of the outer seg-

ment with a longitudinal arrangement. In these eyes the extent of the lamellar system in the short cone is rather poorly developed. As so far noted, the arrangement of lamellae in long cones of *Stolephorus* resembles that of *Engraulis*. Whereas the lamellae in the short cone are perfectly longitudinal with respect to the axis of the cell, those of the long cone are somewhat inclined – largely as a consequence of the cuneate shape of the outer segment. In both the long and short cone, the general longitudinal alignment of lamellae suggests that much of the incoming light on meeting the outer segment passes through or between the lamellae in a direction nearly parallel to their surfaces. This situation is in marked contrast to the perpendicular incidence of light on the disks of cone cells in other vertebrates.

In long and short cones the lamellae are continuous with each other, and in turn with the cell membrane, in an arrangement that is characteristic of vertebrate cones. This continuity of membranes is basically the same as that first described by Sjöstrand (1959*a*, *b*, 1961) in cones of the perch. The pattern of folding of the membranes in the long cone can be envisaged as being derived by rotation of the membrane stacks from a normal cone condition; that is, where the lamellae are transverse and have predominantly closed ends on one side of the stack and open ones on the other, and with the cell membrane overarching those portions with the closed ends. The long cone can be related to this condition by envisaging a turning of the stacks through almost 90°. If, in such a hypothetical condition, each lamella is closed on approximately three sides and open on the other, then on rotating the lamellae a situation could be obtained in which the lamellae have closed margins on the temporal and vitread sides and open ones on the nasal face of the stack. This agrees with the condition found in fully differentiated long cones where lamellae with closed ends occur on the dorsal and temporal faces and the margins over the connecting structure, but where lamellae with open ends lie along the nasal face of the outer segment.

In the case of the short cones the folding of the cell membrane to form the lamella system is more difficult to interpret. Starting with a hypothetical situation in which the lamellae are transverse and, with the connecting structure lying on the nasal side of the cone, a vertical orientation might be achieved by first rotating the lamellae through 90° in a nasal direction. This would give a condition with vertical lamellae having open margins at the sclerad end of the cone and closed ones vitread. The lobes of the outer segment could then be envisaged arising as sclerad outgrowths of the dorsal and ventral ends of this first formed outer segment portion. Such a sequence of development could account for the vertical orientation of lamellae and the presence of the two outer segments lobes.

The system of vesicles found along the margins of outer segment lamellae that have open ends is characteristic of the cones in *A mitchilli* and *A. hepsetus*. There appears to be no comparable system reported in the literature for other vertebrate photoreceptor cells. The morphology of this system of peripheral vesicles in our anchovies is not fully elucidated; it may be truly vesicular or, in fact, tubular. As far as we can tell, the peripheral vesicular system arises as an outfolding of the cell membrane, at the point where it links one lamella to the next, but from individual sections it is not possible to determine whether the structures are always detached or sometimes form blebs in direct continuity with the lamellar system.

The disks of rod outer segments in *Anchoa* have the same general appearance as those found in the eyes of other teleosts and of most other vertebrates. Except for a few of the most vitread ones the disks are closed structures in contrast to the lamellae of the cones. The fact that the rod disks often swell during specimen preparation for electron microscopy, behaving as

osmometers, also indicates that they are closed structures. The disks are typically indented by a single longitudinal incision or cleft similar to that found in the rod disks of other fishes (Locket 1969, 1971*a*; Borwein & Hollenberg 1973), and of higher vertebrates (see Steinberg & Wood 1975).

Vesiculation of outer segment lamellae in long and short cones of *Anchoa* is of sporadic occurrence within an individual eye and among different retinae in both the l.a. and d.a. states. In the alligator, Kalberer & Pedler (1963) occasionally found abnormal arrays of lamellar vesicles and tubules in outer segments of cone-like photoreceptors and, after special consideration, concluded that such formations were not artifacts. They tentatively suggested that the incomplete lamellae might be an indication that the 'cones' were becoming extinct because the species had become nocturnal. Other work by Pedler (1965, 1969) supports the non-artifactual nature of vesiculated lamellae. In contrast, Ahlbert (1973) noted vesiculation in the lamellae of both rods and cones of perch fry, with the greatest amount in rods, and interpreted it as a swelling artifact caused during specimen preparation. Eakin (1965) found extensive vesiculation in cone outer segments and attributed this to the greater sensitivity of cones to fixation.

In our material, although rod lamellae may show signs of having swelled during specimen preparation, vesiculation of the kind found in cones is absent. Pedler (1965) has also found in the diurnal gecko *Phelsuma inunguis* that while vesiculation may occur in 'insensitive' outer segments of cones the condition is absent in rods. The fact that vesicles occur in cones, which have open lamellae, rather than in rods, implies that the vesicles are probably not the product of a simple swelling process through osmotic differences between the fixative and intra-lamella space. Furthermore, and in contrast to the results of Eakin (1965), vesiculation occurs when fixation is carried out in glutaraldehyde. Other workers have also shown vesicles amongst lamellae of photoreceptor outer segments in preparations that, in all other respects, have the appearance of being well fixed (see for example, Samorajski, Ordy & Keefe 1966). Vesiculation of cone lamellae in glutaraldehyde fixed eyes of *Necturus* was reported earlier by Brown, Gibbons & Wald (1963) who stated that there was good reason for believing that the vesicles were something more than a fixation artifact. On the other hand, recent experimental work on the effects of fixation of rod outer segments indicates that, at least in rods, vesiculation of lamellae can be produced by manipulation of the tonicity of the fixing solutions (Falk & Fatt 1973; Jones 1974). Jones found rod outer segments, and to a lesser extent cone outer segments, fixed only with glutaraldehyde that showed shrinkage artefacts which were dependent on the concentration of buffer used.

(ii) *Inner segment*

The ellipsoids of the cones of *Anchoa* show some features that are typical of vertebrate cones, especially those of fishes, and also some unusual structures.

The accessory outer segment (Engström 1963*a*) or lateral sac (Fineran & Nicol 1974) of the long cone resembles that found in the eyes of many other fishes and originates close to the base of the connecting structure. Observations on the occurrence and structure of the lateral sac in teleosts are summarized by Borwein & Hollenberg (1973) and Fineran & Nicol (1974). In the species of *Pseudolabrus* (Labridae) that we examined a single lateral sac was found in all four types of cone cells, whereas in *A. mitchilli* and *A. hepsetus* a lateral sac is wanting in the short cone. A lateral sac is also absent in the rods and this is in agreement with our observations in *Pseudolabrus* (Fineran & Nicol 1974). Our studies on the eyes of *Engraulis mordax* and *Stolephorus*

sp. also indicate that a lateral sac is present in the long cone but absent in the short cone and in the rods. It seems that while lateral sacs are characteristic of certain cones they are not always present and a lateral sac in rods is doubtful, or at most, uncommon.

It is well established that the connecting structure of the vertebrate photoreceptor cell is derived from a cilium, but one in which the central pair of axonemal filaments (microtubules), so characteristic of motile cilia, is normally absent. In *Anchoa* the rods show a typical connecting cilium with the root portion corresponding to a centriole, or basal body, and the shaft to the ring of 9+0 doublet axonemal filaments. The cones, on the other hand, have a connecting structure which superficially appears different, but which on close examination has features indicating its origin from a cilium. The root portion of the connecting structure can be equated to a centriole where the typical 'pin wheel' arrangement of triplets has been lost. The corner thickening material may be reduced triplet portions, as it is from these that the short filaments found in the stalk arise. The homology of the fibrillar-like partitions between the corner thickenings is uncertain. We have not found any structures in the rods and cones that could be identified as part of a fibrillar rootlet system associated with a centriolar basal body. Compared with the rod, the filaments in the stalk of the cones are considerably reduced within each bundle and seem to consist of but a single filament. In other vertebrate photoreceptors a reduction in the number of filaments may occur in the distal portion of the cilium where it runs alongside the outer segment (Brown *et al.* 1963). In *Anchoa* modification of the stalk portion has proceeded further to the point where, in some cones, the radial distribution of the bundles is uneven and the number of bundles has decreased. From such observations as these we believe that the connecting structure of the long and short cones represents a reduced and highly modified ciliary structure.

Calycal processes (Cohen 1963) or 'dendrites' (Brown *et al.* 1963) are structures typical of many vertebrate photoreceptor cells, although they are not necessarily of universal occurrence throughout the vertebrates (Brown *et al.* 1963). The first electron microscope report of these structures was by Sjöstrand & Elfvin (1957) in the rod of the toad. Calycal processes form a palisade of regularly spaced tapered structures surrounding the outer segment; in this respect, the two separate groups on the nasal and temporal faces of the cones in *Anchoa* represent an unusual arrangement. However, observations on the differentiation of the long cone indicate that such a grouping of calycal processes is a secondary condition derived during ontogeny from a continuous ring. Calycal processes appear to be absent, or if present poorly developed, in the rods of *Anchoa*.

The ellipsoids of vertebrate photoreceptors characteristically contain numerous mitochondria (Sjöstrand 1953). Frequently these mitochondria are large and well differentiated in terms of cristae development. In *Pseudolabrus*, for example, we found a condition where the cristae exhibited a convoluted appearance, with each crista having a row of lateral branches on one side inter-digitating with those of adjacent cristae (Fineran & Nicol 1974). The cristae in mitochondria of cone ellipsoids of *Anchoa* mostly form a system of closely packed tubules. A noteworthy feature is the development of a variously expanded perimitochondrial space and the occurrence, although sporadic, of tightly wound masses of membranous material developed from the envelope of the organelle. Recently, we have also found mitochondria with an expanded perimitochondrial space in cone ellipsoids of *Engraulis mordax* and *Stolephorus* but, apart from these observations on anchovies, mitochondria with extensively expanded perimitochondrial spaces are, to our knowledge, unknown among other animal cells.

In lower vertebrates there is frequently a gradient in the size and differentiation of

mitochondria towards the outer end of the cone ellipsoid whereas in the rods of most animals the population of mitochondria is more homogeneous (Ishikawa & Yamada 1969). Among fishes such a gradient of mitochondria in cones has been shown, for example, by Berger (1966) in the guppy and by us in double and short single cones of *Pseudolabrus* (Fineran & Nicol, 1974). The general uniform size of mitochondria throughout cone ellipsoids of *A. mitchilli* and *A. hepsetus* is, therefore, something of an exception.

Myoid folds, fins or inner segment ridges have been reported in photoreceptors from a variety of vertebrate avascular retinae (see discussion in Borwein & Hollenberg 1973). The structures typically form an intermeshing system with the sclerad directed microvilli-like processes that arise from the Müller cells at the level of the external limiting membrane. It has been suggested that myoid folds are concerned with transport and nutrient flow between the photoreceptors and the Müller cells (see Dunn 1966).

Structures resembling microtubules and small bundles of microfilaments are occasionally observed in the thinnest sections cut longitudinally through the inner segments of the long and short cones. These structures may be more widespread in our material but were not observed because we have generally used sections thicker than are desirable for studying these components. Microtubules and microfilaments have been reported by a few other workers (for example Locket 1973) in the peripheral zone of cytoplasm surrounding the mitochondria of the ellipsoid and myoid. There are suggestions in the literature that microfilaments and microtubules are implicated in cell movements and cytoarchitecture (for reviews see Wessells 1971; Bardele 1973). The occurrence of these structures within the inner segments of photoreceptors that undergo photomechanical movements may provide a structural basis for these movements and for maintaining the shape of the cell. Burnside (1975) and Warren & Burnside (1975) have outlined some recent work on microtubules and actin filaments in relation to photomechanical movements in teleost retinae. It is also of interest to recall our earlier work on *Pseudolabrus* (Fineran & Nicol 1974) in relation to the occurrence of microtubules in photoreceptors that undergo photomechanical movements. The photomechanically active long cone in these fishes has a well developed system of microtubules whereas the stationary short single and double cones appear to be devoid of the structures. The existence of microfilaments in photoreceptors of *Pseudolabrus* was not investigated. Structures fitting the description of microtubules were also recorded by Engström (1963*a*) in long cones of other labrid fishes but the photochemical movements of their various cones were not completely understood.

The contact zone developed between the ellipsoid of the short cone and the corresponding adjacent area of the long cone ellipsoid is structurally similar to that described first by Engström (1963*a*) in double cones of labrids and later by Stell (1965) in goldfish, Berger (1967) in neonatal and adult double cones of guppy and Ahlbert (1973) in developing double cones of perch. Our observations on the organization of the contact zone in other labrid fishes are in agreement with those described by Engström (1963*a*). The most characteristic feature of the contact zone between cones is the development of a single sheet of cisterna within each cell situated immediately adjacent to the opposed cell membranes. Most workers consider that the subsurface cisterna within the ellipsoid is an entire sheet without fenestrations, although perforations may appear within it as the cisterna approaches the myoid region (Ahlbert 1973; Fineran & Nicol 1974). However, our observations on *A. mitchilli* and *A. hepsetus* indicate that small scattered fenestrations may, in fact, also be present within the cisterna throughout its extent within the ellipsoid.

One of the problems in reconciling the organization of the contact zone with communication between the cells is that the spacing of the opposed cell membranes is generally wide; this is in contrast to those situations where membrane junctions are developed between cells. However, our observations on the cone rows of *Anchoa* indicate that scattered throughout the contact zone, and more especially towards its lateral margins, there are small areas of what appear to be membrane-to-membrane contacts or adherent spots. In many preparations these contacts are broken, as a result of specimen preparation, but their existence can often still be inferred from the presence of small evaginations of the opposite cell membranes. Possibly such membrane-to-membrane contacts provide a site for a lowered electrical resistance between the cones. Alternatively, these local contact spots might provide sites for the structural 'buttoning' together of the cones of a row; such a system would, no doubt, be advantageous where the cones undergo radial extensions during photomechanical movements of the eye. In the case of double cones of *Pseudolabrus*, we did not observe these contact points between the cones and this may possibly be related to the fact that the double cones here undergo little or no photomechanical movement.

Besides a possible role in the conduction of stimuli or metabolites the linking of cones has certain optical implications. As there is no refractive discontinuity between the cones of a row light should pass through them as an entity. In the case of retinae with double cones, each pair might function as an entity sampling the same region of the visual field (Cohen 1963), but in the eyes of *Anchoa* each short/long cone combination is presumably integrated with other short and long cones throughout the row.

The relation between photoreceptors and the external limiting membrane region of the Müller cell in fishes has until recently received little attention (Uga & Smelser 1973). Cell junctions characterized by an increased electron density of the membranes and adjacent cytoplasmic ground substance occur in this region. Uga & Smelser (1973) found in many animals, including fishes, that the external limiting membrane was composed of *zonulae adhaerentes*, but in carp and top minnow there were also extensive gap junctions between the Müller cells. In carp desmosomal connections (*maculae adhaerentes*) were also present forming part of the external limiting membrane. In anchovies the junction of the photoreceptors with the Müller cells has certain resemblances to an intermediate junction (either *fascia* – or – *zonula adhaerens*) but the tiny contact areas tend to confuse the picture; possibly these may represent a small localized nexus scattered within the intermediate junction.

### (*e*) *Structure of the cones in relation to the way in which they might receive light*

Except in anchovies, the lamellae/disks of the outer segments in vertebrate eyes are oriented perpendicular to the long axes of the photoreceptors. The photosensitive pigment molecules are oriented with their longitudinal axes, and hence their chromophores, parallel to the disk lamellae, but within the plane of the lamellae they are probably arranged randomly. Outer segments show no dichroism to light entering them parallel to their long axes, i.e., in a physiological direction, but they are dichroic when illuminated in a direction normal to the optic axis, i.e., perpendicular to the long axis of the outer segment. When the outer segments are illuminated with polarized light normal to their long axes, absorption is greatest when the direction of vibration (the *e*-vector) is transverse (perpendicular) to the longitudinal axis of the photoreceptor, and least when parallel to it (Kirschfeld 1969; Liebman & Entine 1968; Liebman 1972).

The lamellae of the cones in the eye of *A. mitchilli* and *A. hepsetus* are oriented mostly parallel to the long axis of the outer segments. If the visual pigments in the cones are arranged within the membranes as they are in the goldfish, for example, then it may be expected that the cones will be dichroic to light entering in the physiological direction. If the light is linearily polarized, an outer segment will more effectively absorb a train of light waves when the planes of the lamellae lie in the planes of vibration of the *e*-vector. Absorbance in a cone outer segment will depend upon the angular rotation between the planes of the lamellae and the direction of vibration of the light. The structural organization of the cone unit indicates that *Anchoa* possesses a two tiered system of dichroic membranes, consisting of two adjacent stacks of parallel lamellae below, on which is superposed a stack of parallel lamellae oriented orthogonally to those of the stacks below. The system, as described, is one that has the apparent capability of analysing plane polarized light according to the direction of the *e*-vector.

No system resembling that of *A. mitchilli* and *A. hepsetus*, to our knowledge, has been described in the vertebrate eye but analogous ones occur in invertebrate phyla where the absorbing systems consist of rhabdomes made up of ordered rows of microtubules (Eakin 1972). In the honeybee, for example, the microvilli of the eight retinular cells are aligned in two directions perpendicular to each other; this arrangement occurs along the length of each rhabdome (Goldsmith 1962). Similarly, in the squid the rhabdomes of the two retinular cells have their microvilli perpendicular to each other along the axis of the rhabdome (Zonana 1961). In decapod crustacea, however, the microvilli of a given rhabdomere are organized into many layers that are interleaved at right angles with villi of adjacent rhabdomes (Eguchi & Waterman 1966). Both in decapod crustacea and cephalopods the two directions of the microvilli are parallel to the vertical and horizontal axes of the animals. Their directions coincide with the maximal effectiveness of the *e*-vector of the light stimulus. It has been concluded that the two orientations of the microvilli in the rhabdomeres produce a two channel analyser. This function probably depends upon the dichroism of rhodopsin incorporated in the membranes of the microvilli.

As a further comparison, the two tiered system of dichroic lamellae in *A. mitchilli* and *A. hepsetus* is incorporated in a well developed system of cone rows that run vertically in the eye (relative to the axis of the fish). Each vertical row of cones is a series of ordered polycones which are made up of cone units or triplets. The lamellae of the long cone are essentially horizontal while those of the short cone are vertical to the body axis. In these anchovies, therefore, as in the crab, the two directions of the membrane system (lamellae and microvilli, respectively), are parallel to the vertical and horizontal axes of the animal.

The reflecting platelets of the pigment epithelium cells that form an inverted V over the cones lie in the same vertical rows as the polycones (Fineran & Nicol 1977). The planes of platelets lie approximately 10°, on either side, to the horizontal axes of the cones (in the central regions of the eye). Two stacks, from adjacent pigment epithelium cells, lie over the sloping wedge-shaped face of a long cone outer segment and extend down between the two limbs of a short cone. Therefore, each cone unit or each triplet is optically isolated from its neighbours by a wedge of interference mirrors, although at the base of the short cone outer segment (where the lamellae are transversely oriented) there are only a few rows of platelets (figure 48, plate 15) and these may not provide complete isolation.

As a consequence of this organization, trains of light waves vibrating horizontally, parallel to the long cone lamellae, will encounter the reflecting platelets parallel to their surfaces.

Observations on the reflection of polarized light by dissected l.a. eyes of *A. mitchilli* indicate the importance of this light in the operation of the retina. The reflected light is plane polarized and the direction of vibration is perpendicular to the direction of the vertical rows and parallel to the surfaces of the reflecting platelets. For a stack of isotropic dielectrics having the refractive indices of the system under examination, reflected light is completely plane polarized at an angle of incidence of 54° (Denton 1970). The reflecting platelets of the tapetum of the anchovy eye are regularly arranged. If we assume that internal reflection of light rays takes place within a cone outer segment and the critical angle is about 72°, then the light emerging from the cones falls upon the platelets at angles of incidence greater than 58°. By simple geometry it can be shown that axial rays striking the platelets at about 58° will be successively reflected from the surface at progessively smaller and then greater angles, emerging through the cones in approximately the same direction as entry. Since the rays are reflected several times successively at high oblique angles, it would be expected that incident light reflected from their surfaces would be plane polarized to a considerable degree.

If the light reflected from the surfaces of the platelets is plane polarized, then the vibration direction of the reflected light would be horizontal. We may postulate that some proportion of the vertical component of incident light is absorbed by the short cones, some proportion of the horizontal component is reflected by the platelets and part is again absorbed by the long cones. Light that is reflected obliquely and transversely across the long cone outer segments is already plane polarized; presumably this light is not absorbed by the short cones. The reflector, therefore, reinforces selective absorbance by the cones. The restriction of the reflecting platelets to essentially a horizontal plane introduces one element of asymmetry into the polycone system. Presumably, they increase absorbance in the long cone outer segment whose lamellae are approximately horizontal, and which is the analyser of horizontally polarized light.

We consider that the guanine platelets reflect light mostly vertically into the cone unit which they surmount. Because of the angular arrangement of the platelets, light reaching them will be reflected several times diagonally across the long cone outer segment in a transverse (and ultimately vitread) direction. In a system of 10 superposed reflecting platelets, organized as 1/4 $\lambda$ films, a reflectivity of 99 % can be achieved by constructive interference. The wave band of course is restricted. The parameters afforded by the electron micrographs point to reflexion centred at about 600 nm. Platelets in fresh preparations exhibit a medley of colours, probably the result of some variability of spacing between stacks. Variation of spacing within a stack can cause the reflexion curve to be broadened. The overall result will be a broad spectrum of reflexion within one cone unit (Fineran & Nicol 1977).

We hypothesize from the structural arrangement that the cone units are two channel analysers for determining the plane of vibration of linearily polarized light. A single cone unit contains two analysers orthogonally disposed to one another. From the organization of the cones we may infer that a cone unit is a functional unit for determining the plane of polarization. A point of light of minimal size could traverse the contiguous limbs of two cones and one overlying long cone, i.e., a cone unit or triplet. However, the light that reaches one long cone outer segment has traversed two short cones that are associated with three long cones. Therefore, the minimal size of a physiological unit responding to a small point of light would consist of three adjacent long cones and two associated short cones. Visual pigment and absorption characteristics of the cones in *A. mitchilli* and *A. hepsetus* are unknown.

### (*f*) *Retinomotor activity and function of the photoreceptors in the dark adapted eye*

The above discussion pertains to the possible functions of the cones in the l.a. eye. As in many other fishes, the retinae of *Anchoa* undergo marked changes during retinomotor activity involving radial rearrangements of photoreceptor inner and outer segments, pigment epithelium cell processes and in the latter also a redistribution within the cell of tapetal material and melanosomes (Fineran & Nicol 1977). The retinomotor movements are intricate due to the mass displacement of the rods and the cones, the linked movement of the long and short cones of a cone unit together with that of the associated processes of the pigment epithelium, and the coordinated movement of whole rows of cones – at least over the same area of the retina. However, the various movements do not appear to be completely synchronous. The rows of cones and processes of the pigment epithelium seem to be the first cells to undergo retinomotor activity, followed later by the main movement of the rods. But, unlike the cones, the rods move individually rather than *en bloc*, and it may take some time for all of them to assume the correct position for the new adapted state of the retina (see figure 45, plate 14).

On dark adaptation of the retina rod myoids contract so that their outer segments are bared and become functional. The sensitivity of rods in *A. mitchilli* and *A. hepsetus* is enhanced by a tapetal reflecting layer in the pigment epithelium, composed of guanine crystallites, some needles and stacks of platelets (Fineran & Nicol 1977).

On dark adaptation of the retina cone myoids elongate, pushing the outer segments beyond the level of the rods so that they reach the tapetal layer. The concomitant movement of the cones and cell processes of the pigment epithelium means that the relation between the cone units and the stacks of reflector platelets is maintained. However, despite this constant relation, it seems unlikely that the cones are functional in the d.a. state because of the low intensity of light reaching them. During retinomotor activity there is also a slight displacement between the long and short cones apparently caused through some elongation of the long cone inner segment in the region immediately sclerad to the level of the short cones outer segments. Furthermore, the outer segment lamellae are disturbed as a result of compression radially and vertically in the long and short cones, respectively. We believe that in the d.a. state the stacks of platelets surmounting the cone units contribute to the general back-scattering of light to the rods rather than being involved with any functioning of the cones (Fineran & Nicol 1977).

### (*g*) *Organization of the eye in relation to the mode of life of the fishes*

Anchovies in many areas form a high proportion of the biomass and are a most important food fish for man. Consequently, a great deal of research effort has been given to the fish and to the fishery.

Anchovies are fishes of warm waters, fresh and marine. They generally occur over shallow sandy bottoms in waters that are often moderately to very turbid. *Anchoa mitchilli* is extremely abundant in the bays and *A. hepsetus* in bays and coastal waters of the Gulf of Mexico. Peak movements from the bays to the Gulf occur during early summer and autumn (Gunter 1945; Hoese 1958; Copeland 1965; Miller 1965). In general, anchovies are indiscriminate filter feeders of both phyto- and zooplankton, straining them off with their gill rakers (Hobson 1968); they have also been observed to feed upon other small fish (Hayasi 1967; Diener, Inglis & Adams 1974). In turn, anchovies are preyed upon by fishes, birds, mammals and squid (Okutani 1962). They are mostly pelagic schooling fishes (Messersmith, Baxter & Roedel

1969). It appears that Gulf anchovies, at least, do not undertake extensive migrations, but they may move into deeper waters in winter (Hildebrand 1963).

Vision is a dominant sense in anchovies. Anatomical studies on the brain of *Engraulis mordax* have revealed large optic lobes, a small forebrain and a small cerebellum. The eyes are so large that their median curvatures are adjacent to each other and the small forebrain is displaced dorsocaudally (Schwassmann 1965). Observations on the behaviour of anchovies also show the importance of vision in the mode of life of these fishes. Loukashkin & Grant (1965) have found that *E. mordax* is positively phototactic, preferring light of medium intensities (100 lx). It is capable of discriminating qualitatively between monochromatic (green, blue, red) and white lights, and it distinguishes green from blue. These authors believe that the anchovy's perception of light is a function of wavelength apart from intensity. A variety of cones occur in the eye of *E. mordax*, and the diverse structural types may be correlated with wavelength sensitivity. The eye of *Anchoa* contains mostly cone units, however, and nothing is known about colour vision in these fishes.

Underwater light is partially plane polarized, more than 60 % is linearly polarized near the surface, and polarization decreases with depth. The patterns of linear polarization are related to the position of the sun and the degree of polarization is maximal near the surface $\pm 90°$ from the sun's bearing (Waterman 1954; Ivanoff & Waterman 1958).

Sensitivity to polarized light has been demonstrated in arthropods and cephalopods; in vertical beams of polarized light they generally orientate themselves perpendicular to the plane of polarization (Waterman, 1959, 1966, 1974; Waterman & Horch, 1966). There is now growing evidence that some fish can perceive polarized light, for example, halfbeak, goldfish; the structural basis for this sensitivity is still to be determined. As Waterman (1974) has emphasized, suitably oriented lamellae and dichroism in photoreceptors do not demonstrate polarized light sensitivity, but they do suggest the definitive experiment to discover if such exists.

Assuming that *A. mitchilli* and *A. hepsetus* have polarized light sensitivity, it may be conjectured that they make use of it to orient themselves relative to the direction of the light, to govern their movements, to avoid predators and to locate food. For example, they could find it advantageous in locating and hunting zooplankton, which may themselves be polarosensitive and be orienting themselves on submarine polarization patterns (Baylor & Smith 1953; Waterman 1959). As Lythgoe (1972) has pointed out, underwater illumination has a significant component of plane polarized light resulting from Rayleigh scattering, and large particles such as plankton reduce the amount of polarization. A diffusely reflecting object depolarizes the light it reflects. To a fish having sensitivity to polarized light, such an object will stand out in greater contrast when viewed against a background of polarized light, especially if the path of sight is at right angles to the sun's direction.

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## Explanation of abbreviations used on figures

| | | | |
|---|---|---|---|
| b.c. | short (bifid) cone | me. | melanosome |
| b.c.o.s. | short (bifid) cone outer segment | mf. | microfilaments |
| b.p.c. | bipolar cell layer | mt. | microtubules |
| c | cone | mu. | Müller cell |
| c.l. | cone layer | my. | cone myoid |
| c.p. | calycal process | o.n.l. | outer nuclear layer |
| c.s. | connecting structure | o.s. | cone outer segment |
| e | cone ellipsoid | p.e. | pigment epithelium cell |
| e.l.m. | external limiting membrane | p.s. | reflecting platelet stack of pigment epithelium cell |
| i.n.l. | inner nuclear layer | | |
| l.c. | long cone | r | rod |
| l.c.o.s. | long cone outer segment | r.p. | rod partition |
| l.s. | lateral sac | t.l. | reflecting tapetal layer of guanine crystallites |
| m | mitochondrion | | |

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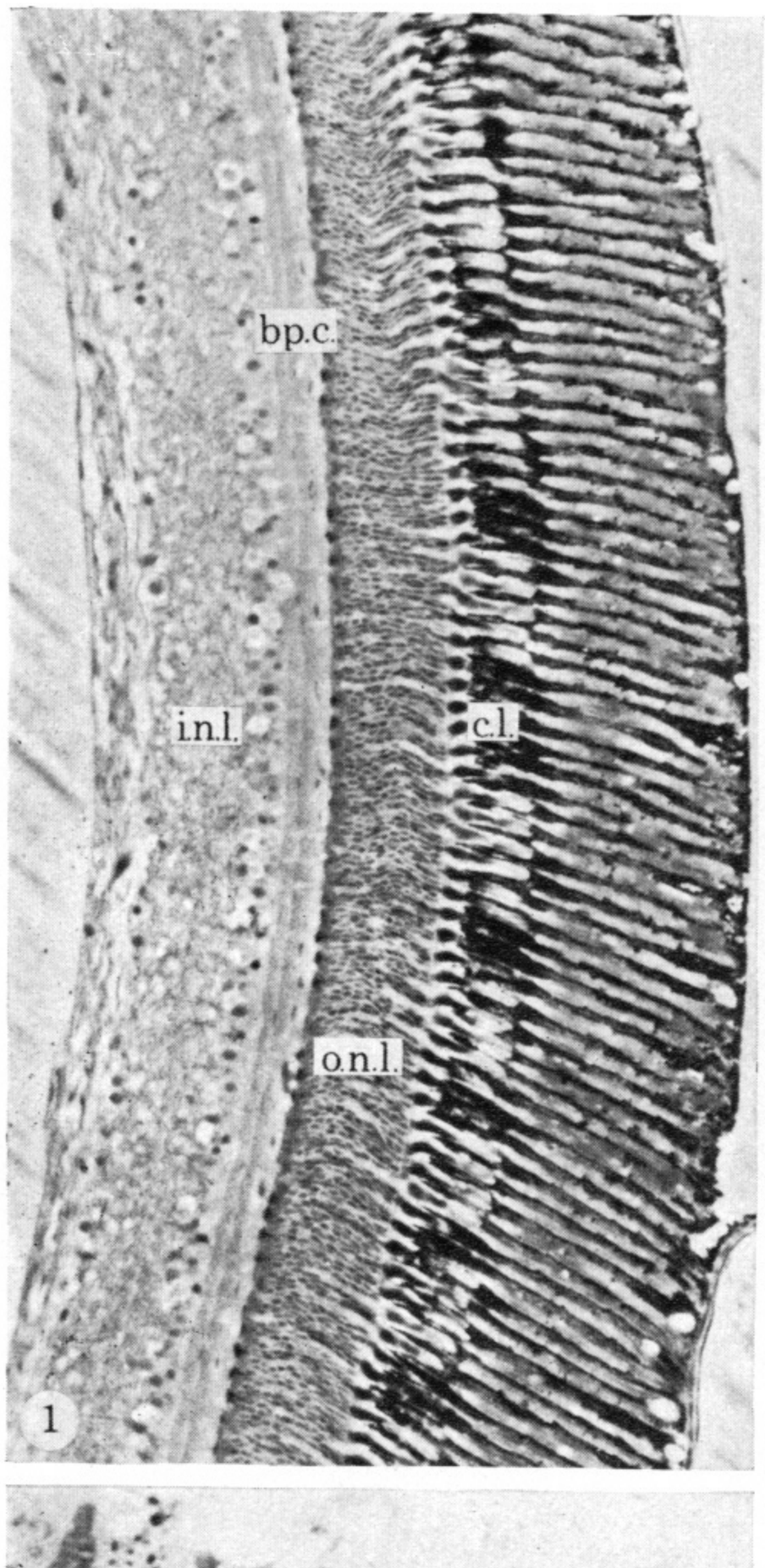


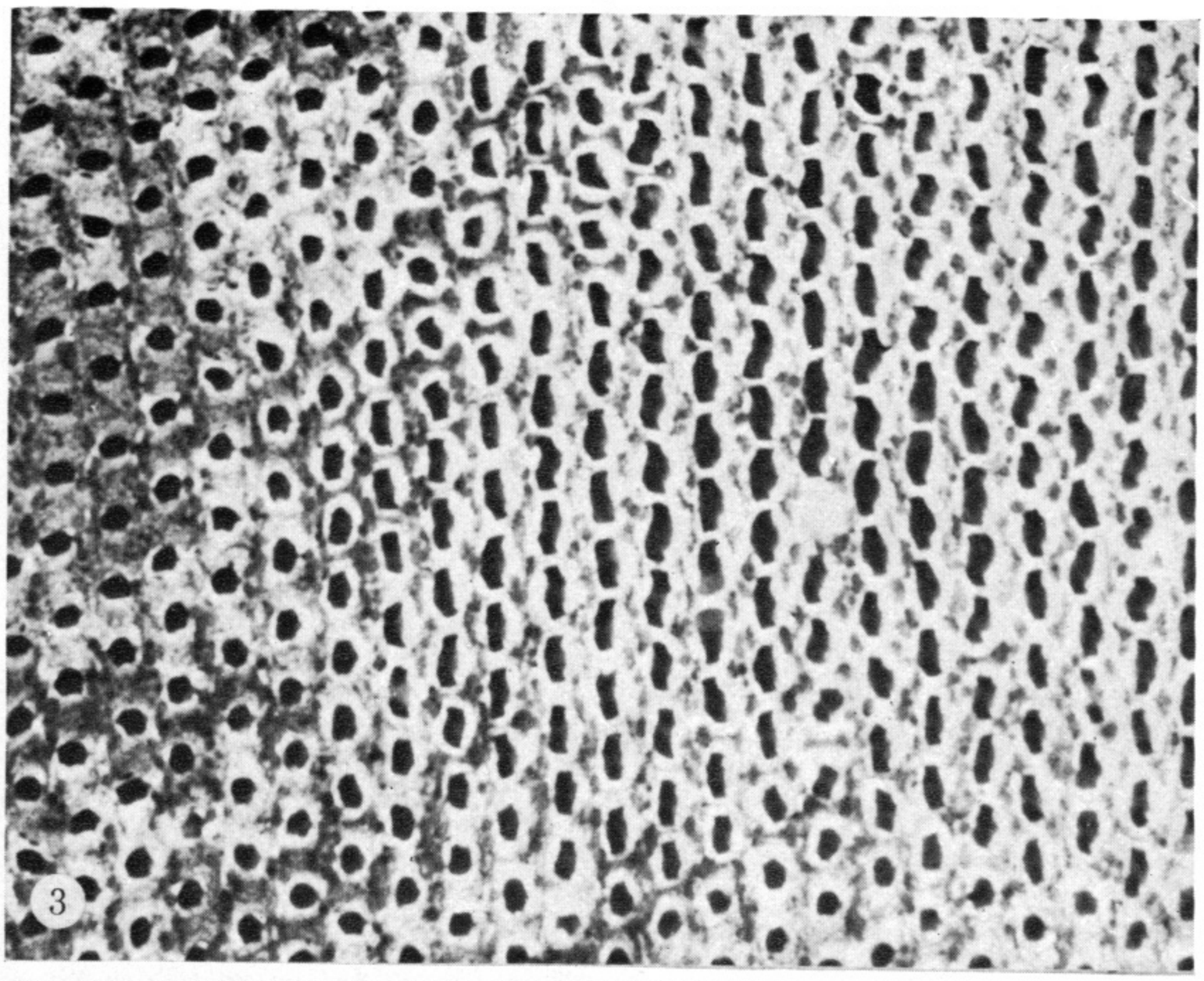


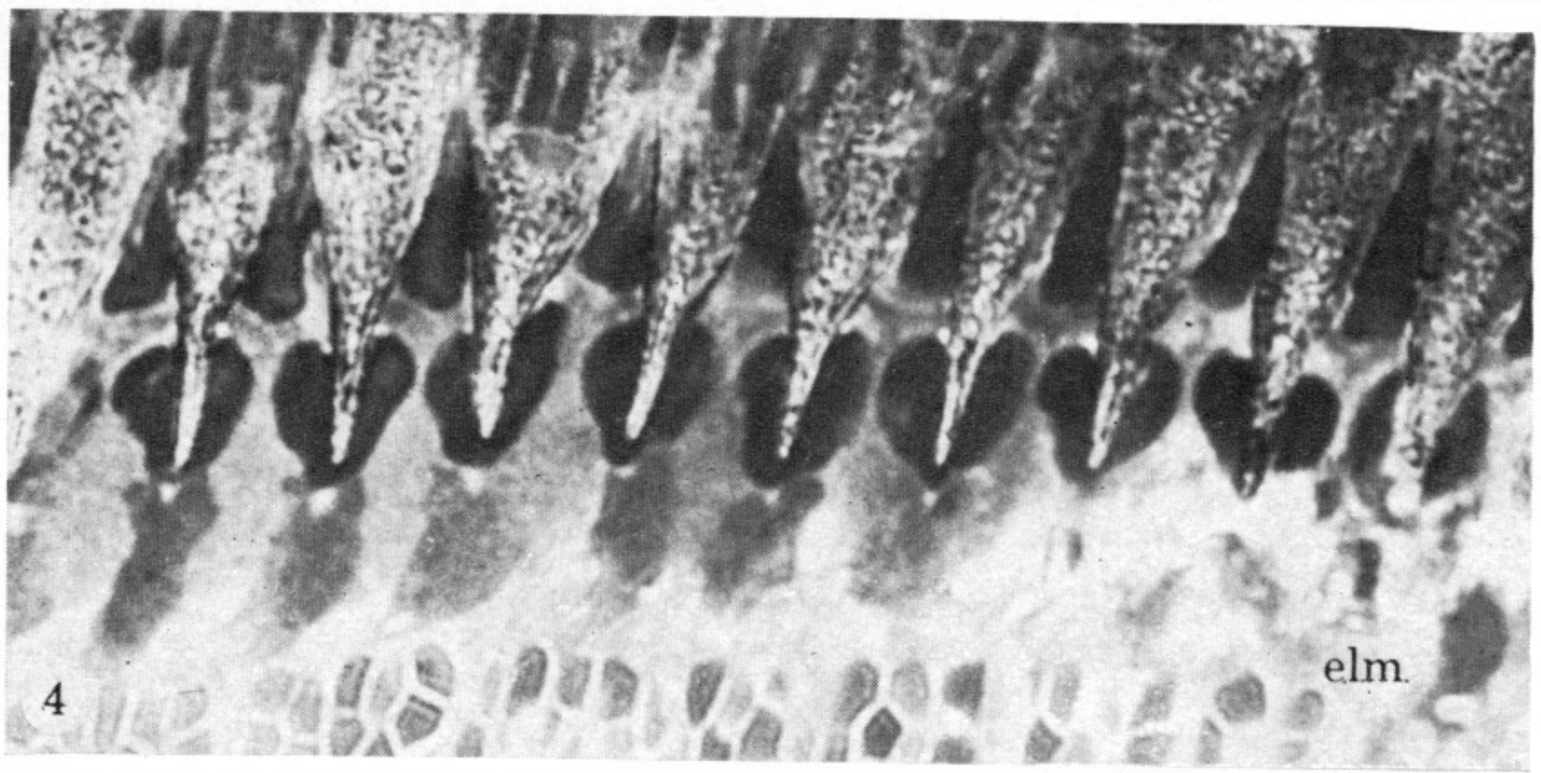


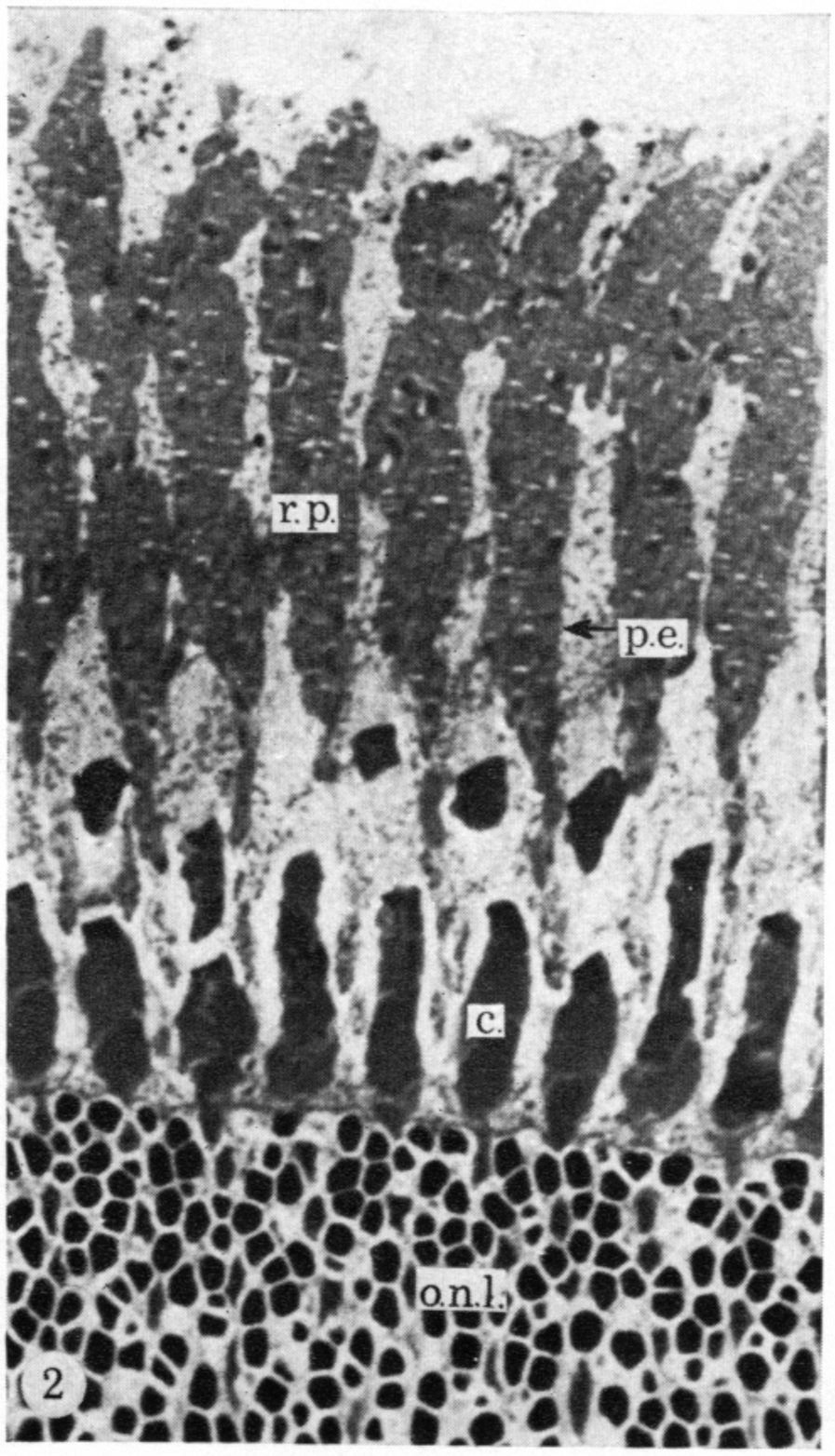


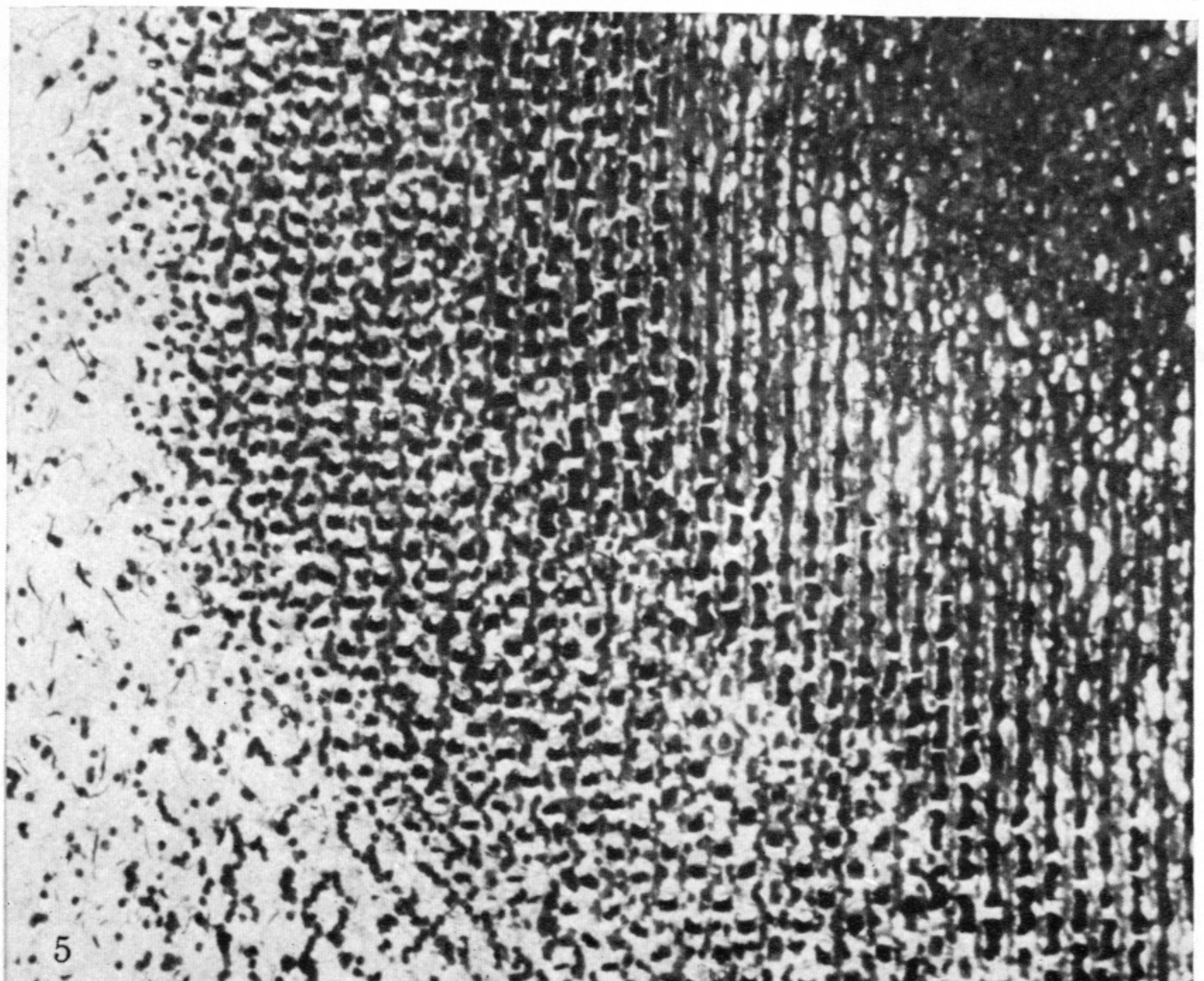


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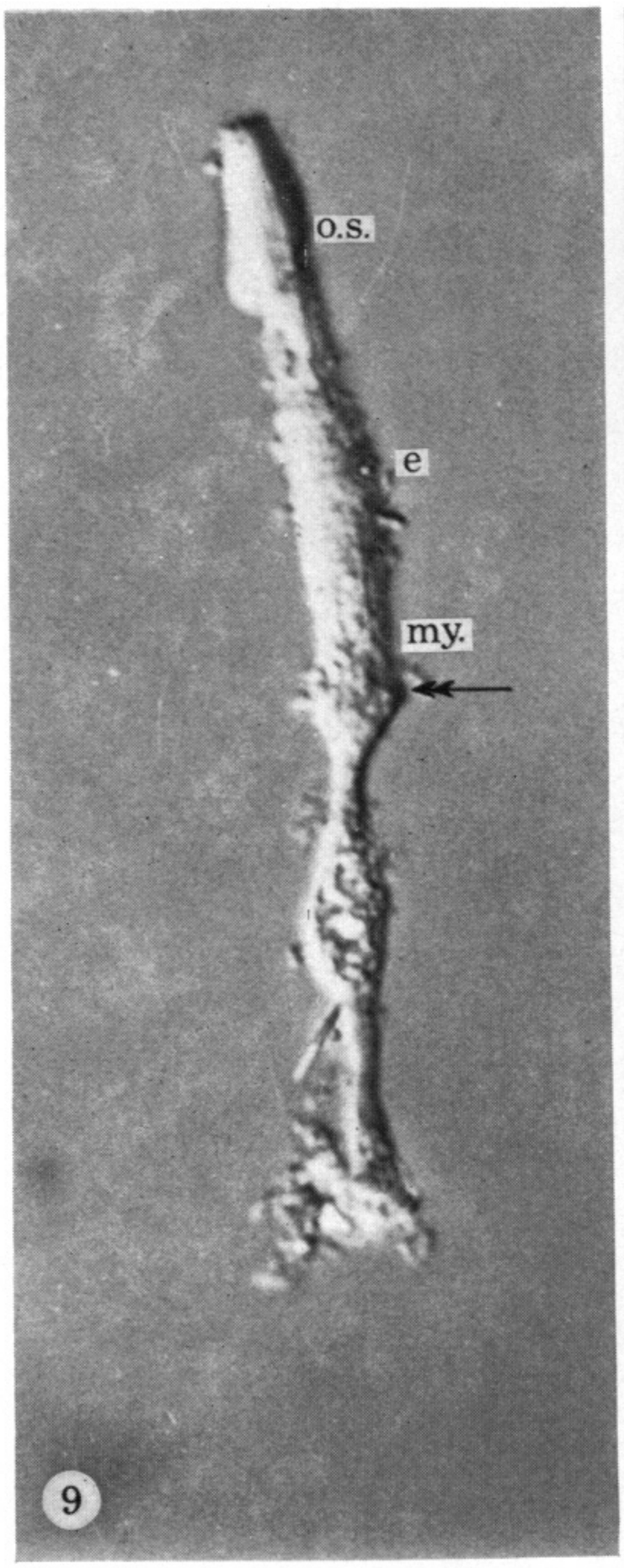


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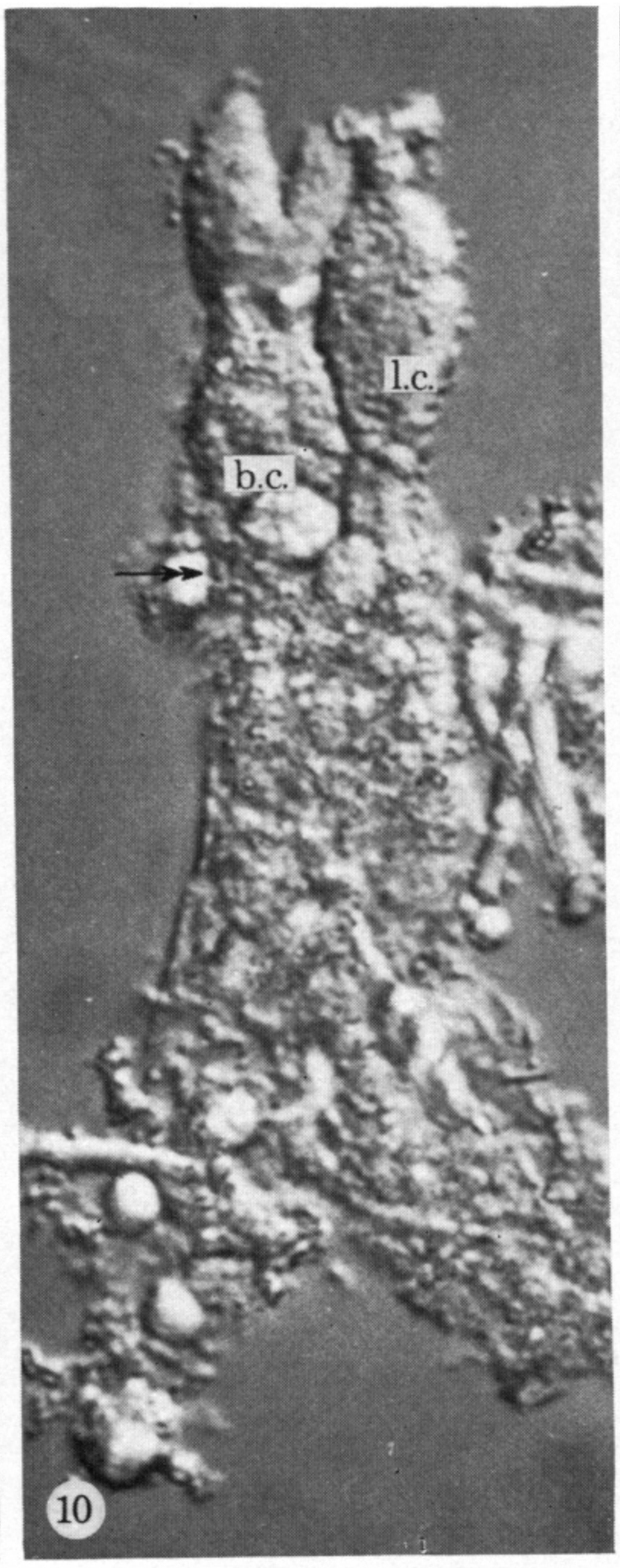


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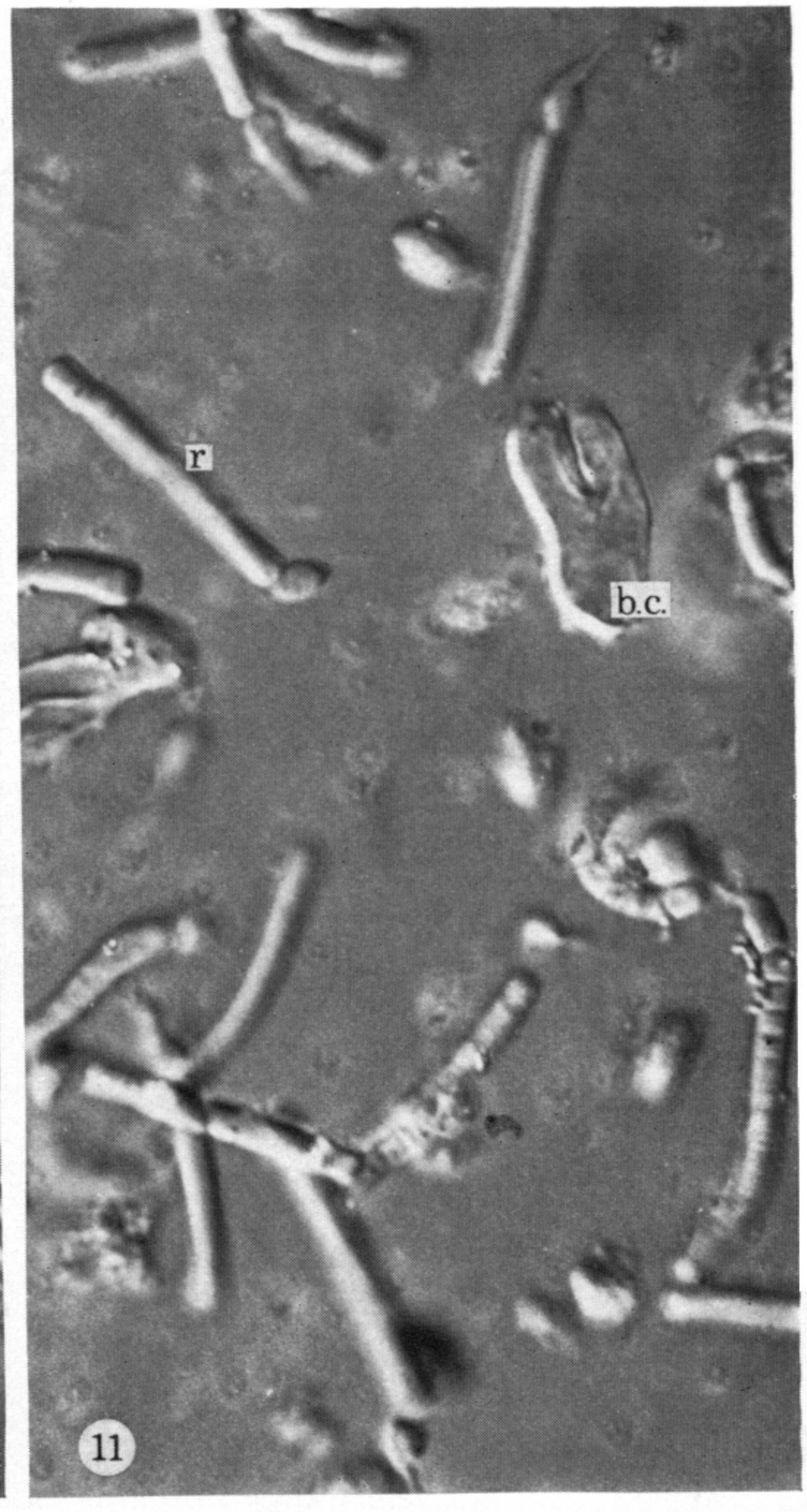


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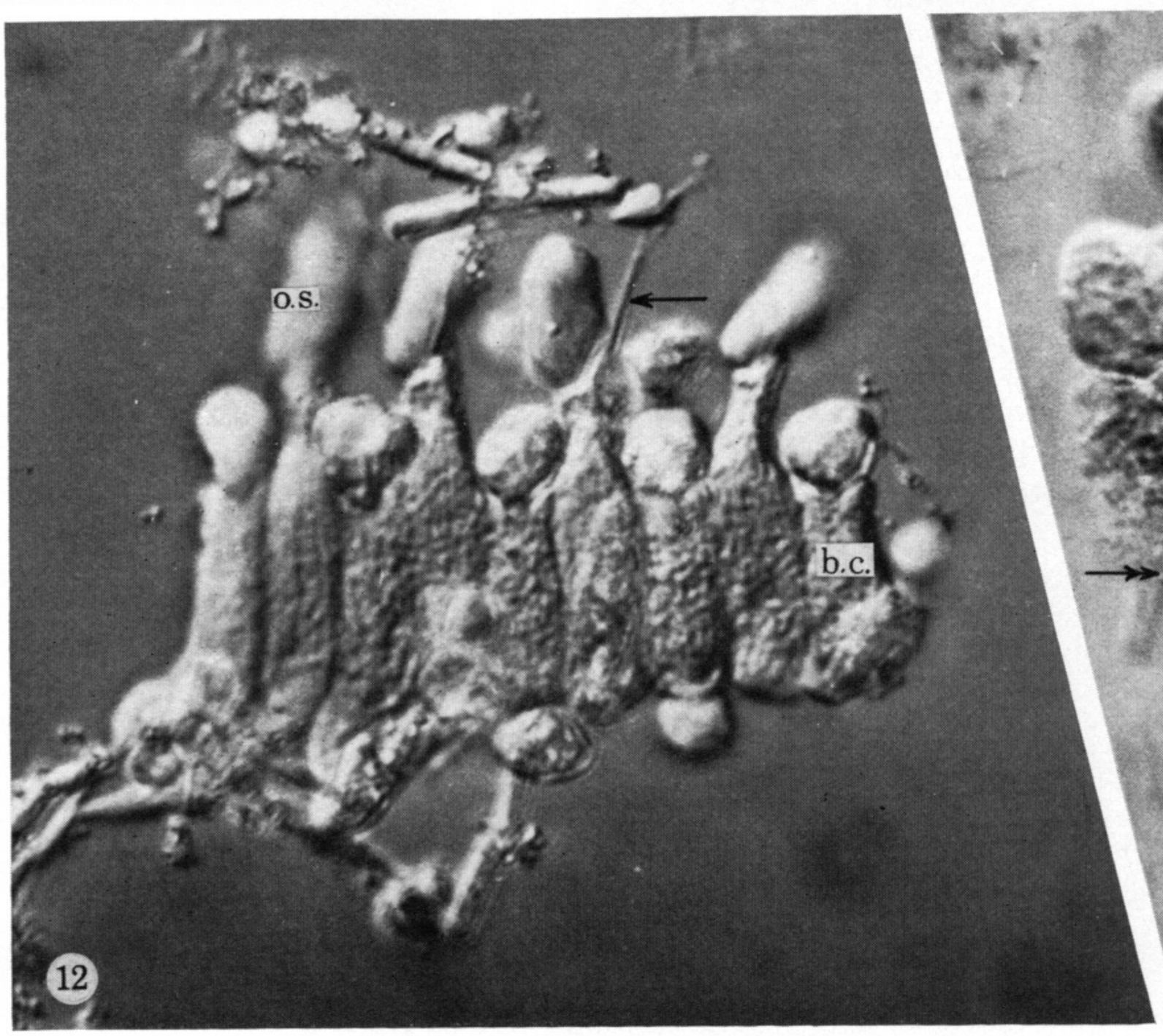


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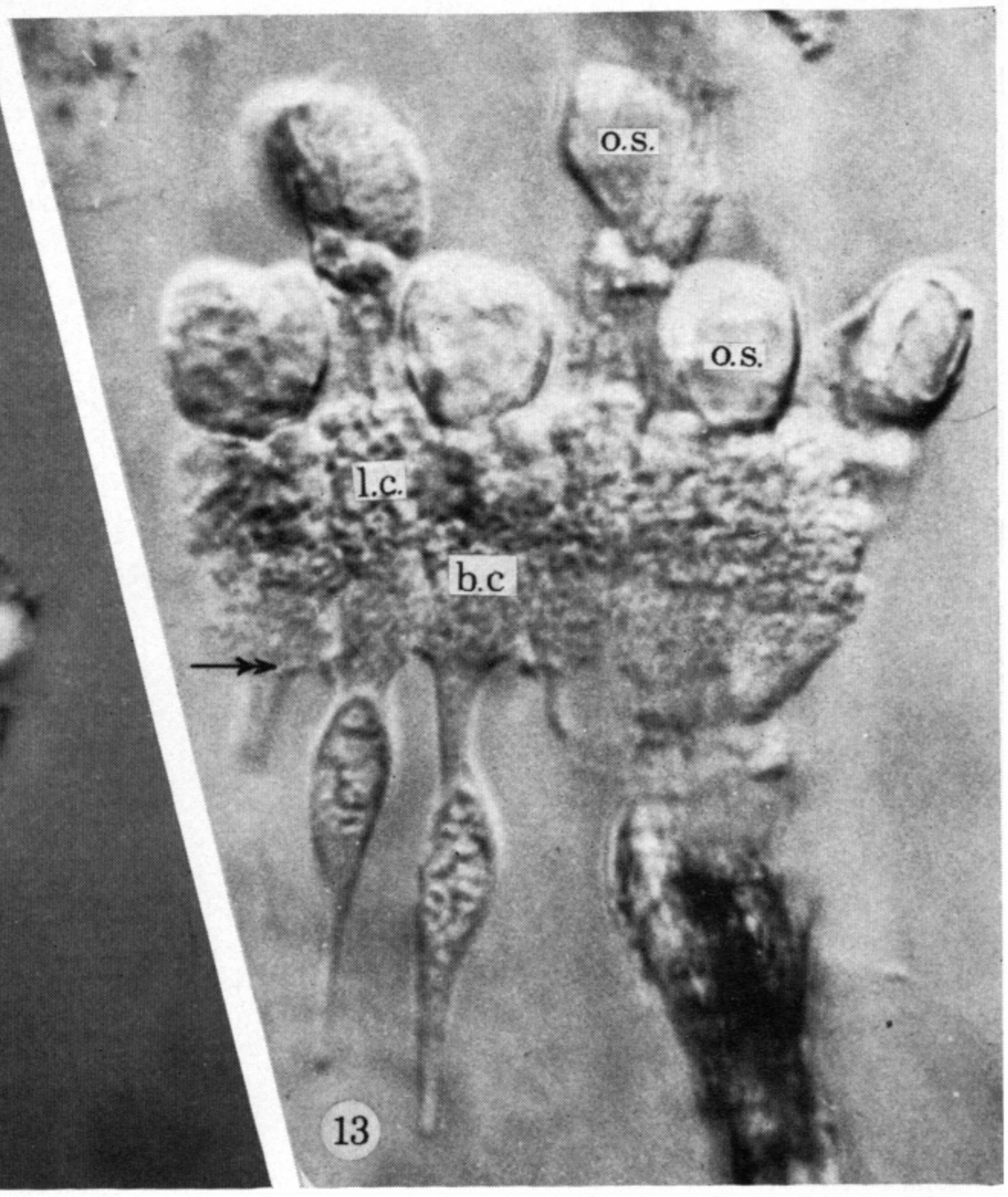


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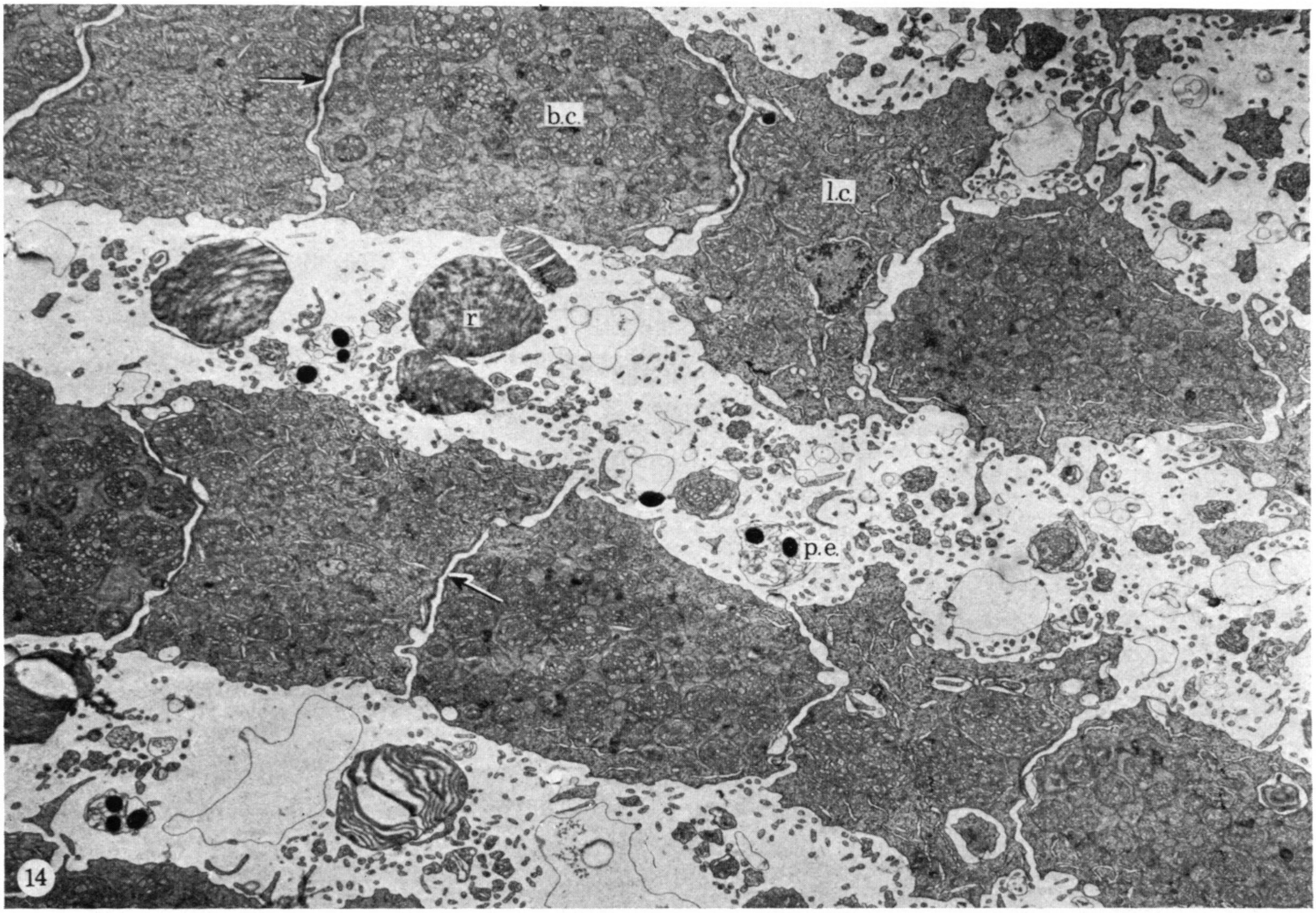
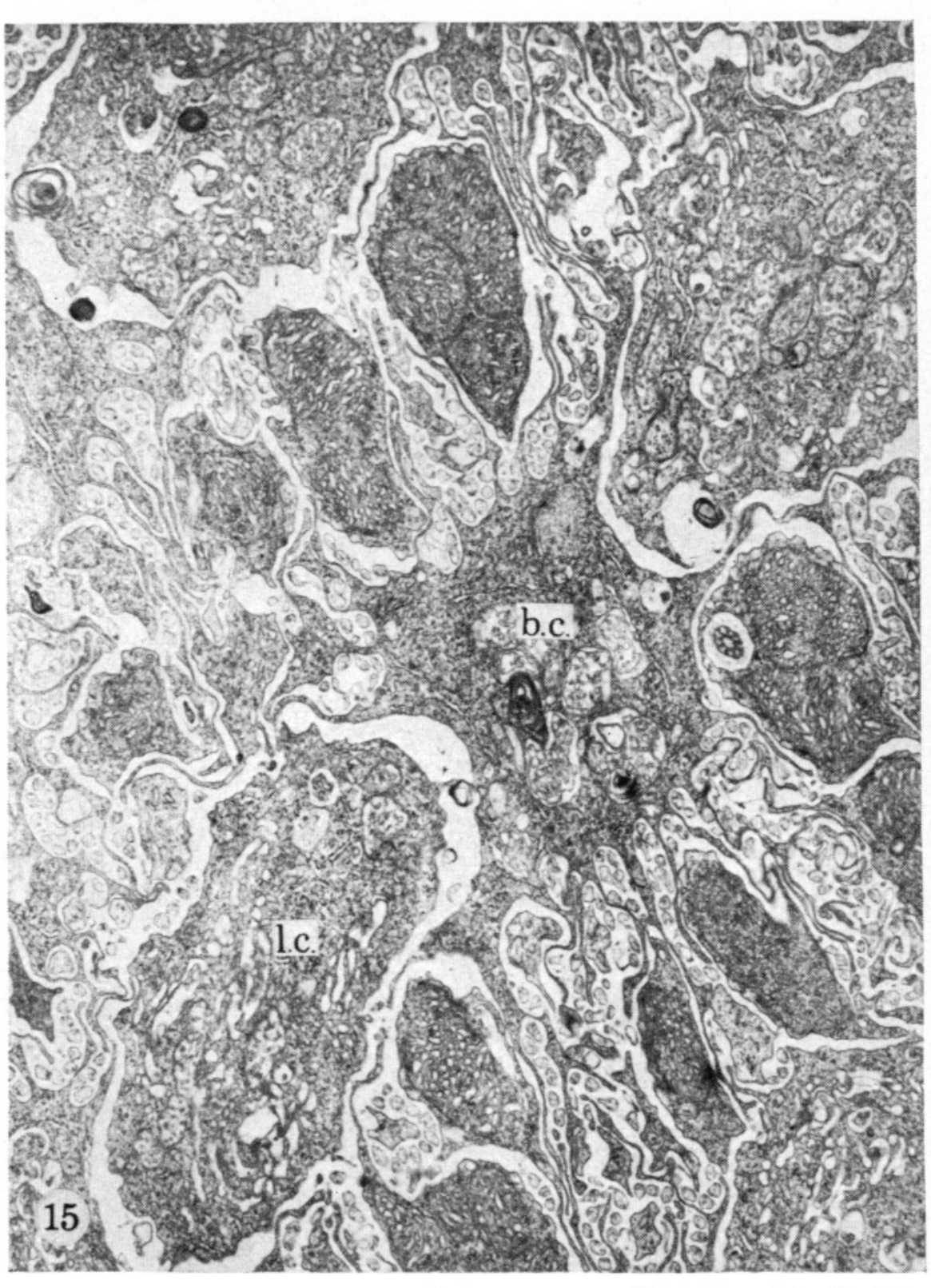
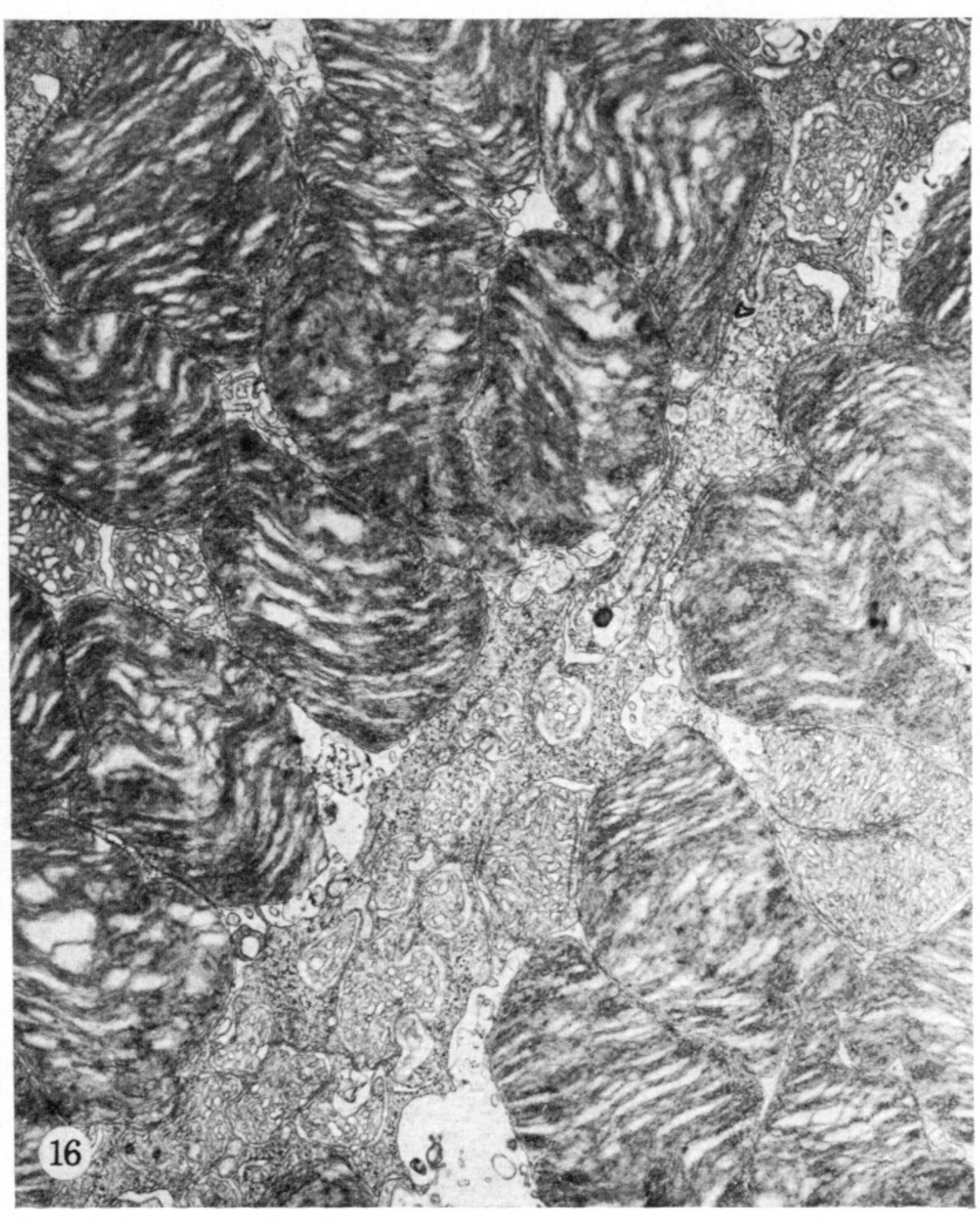
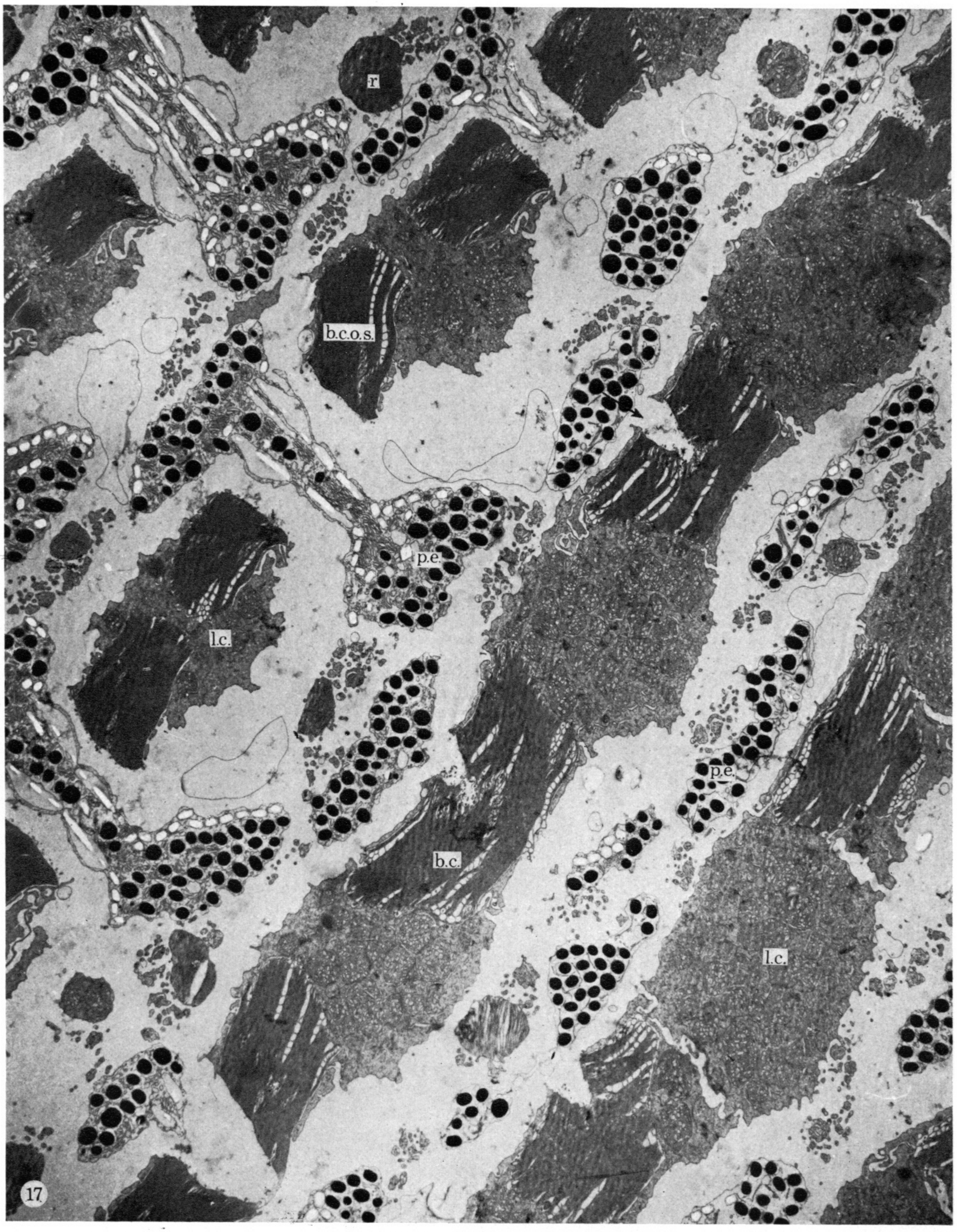
FIGURES 9–13. For description see page 28.




FIGURES 14–16. For description see page 29.

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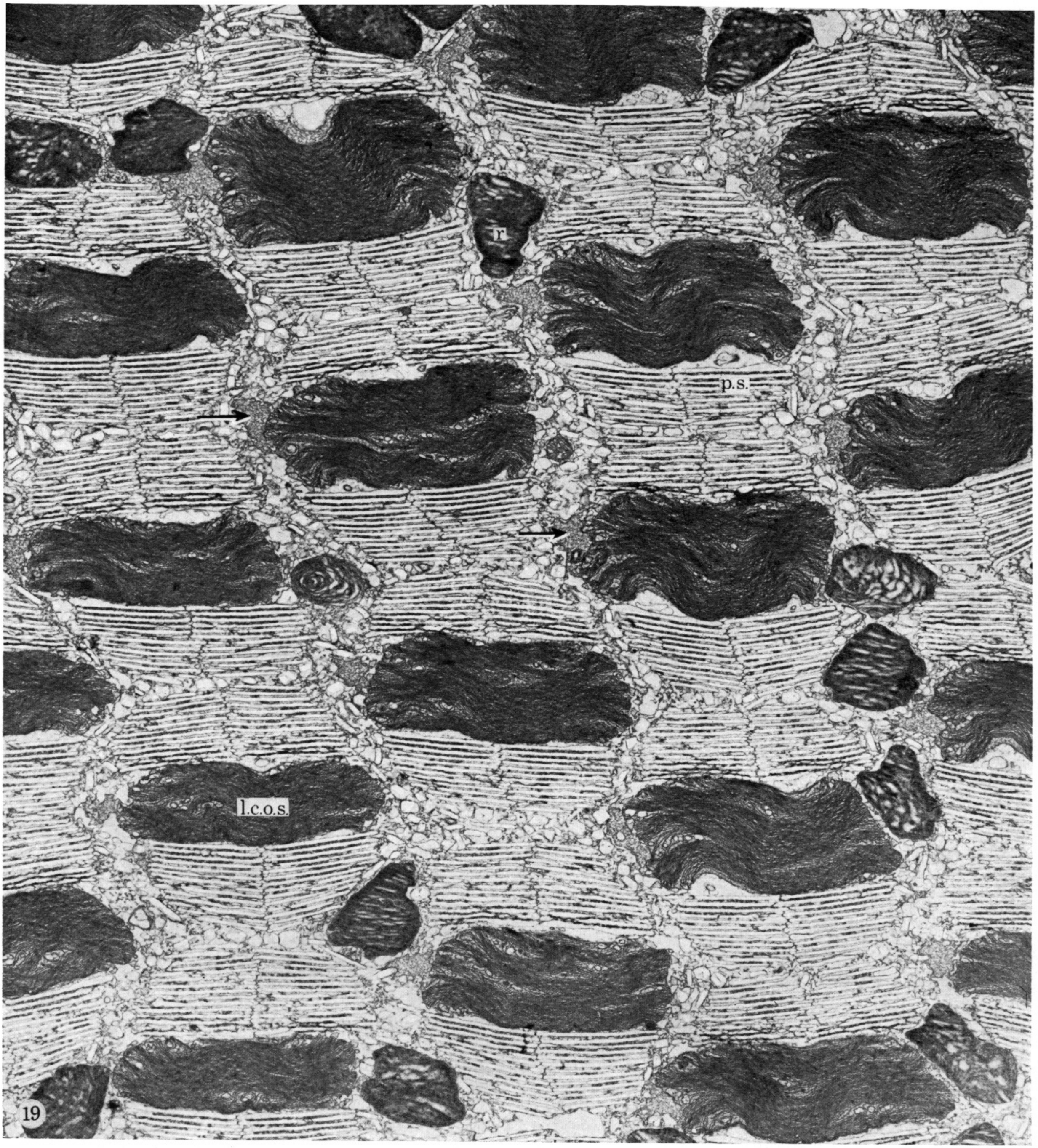


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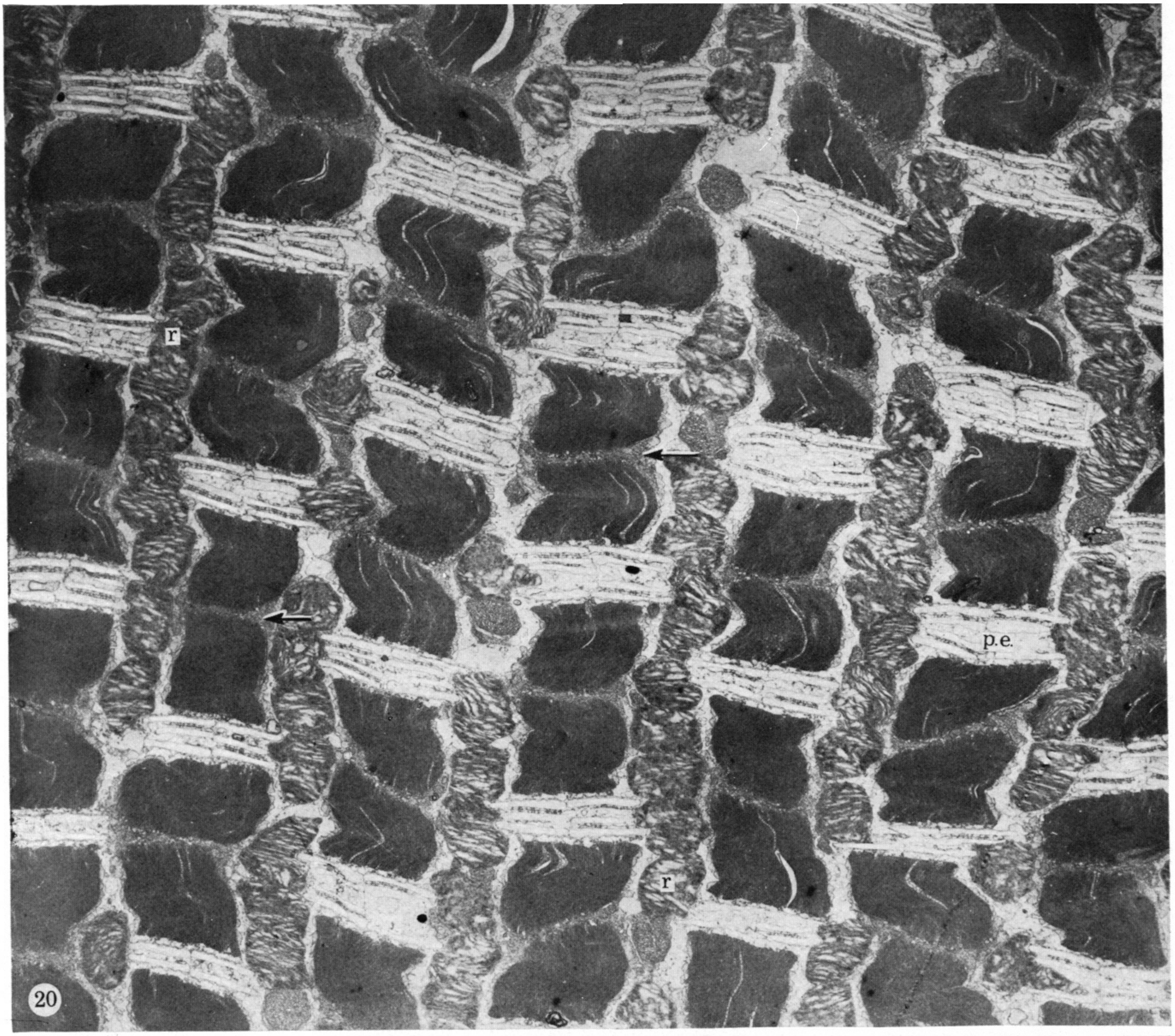
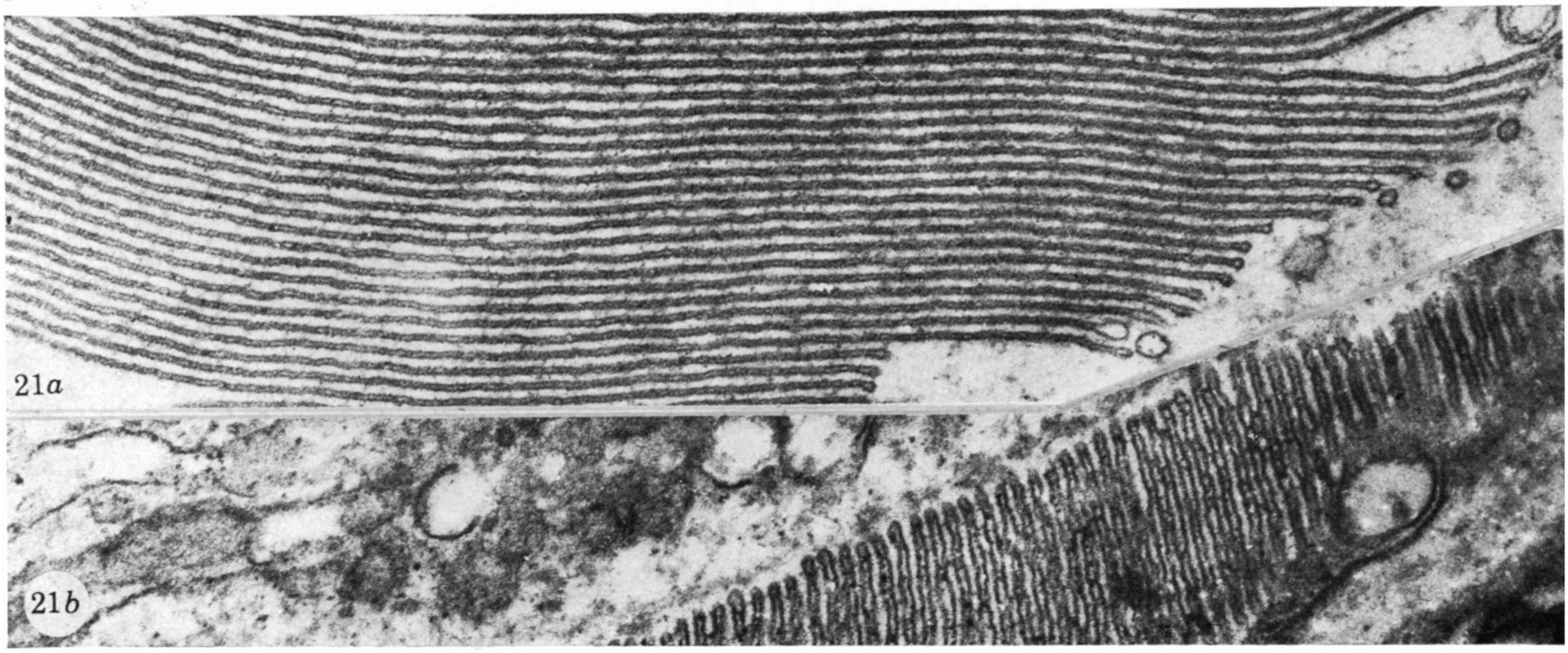
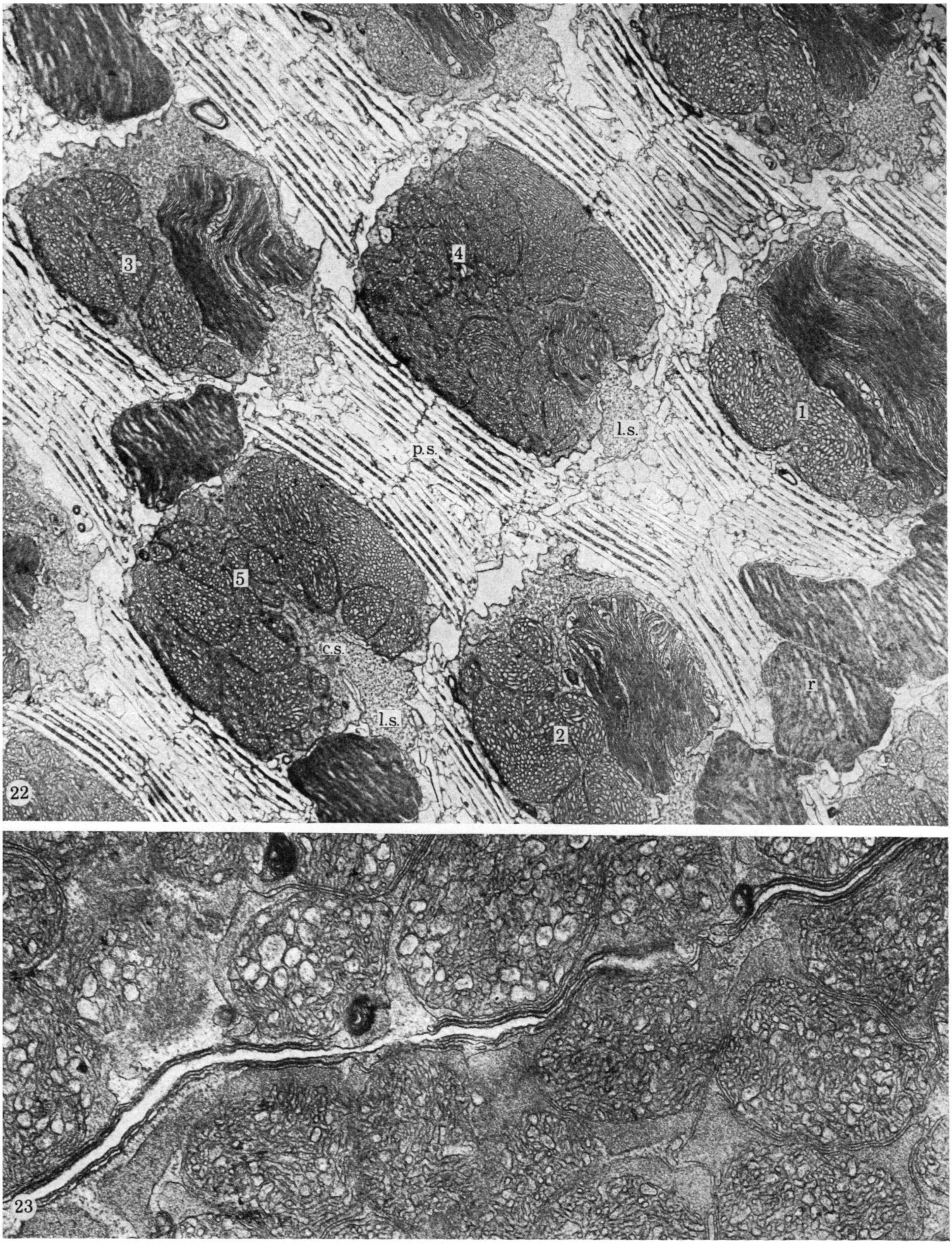
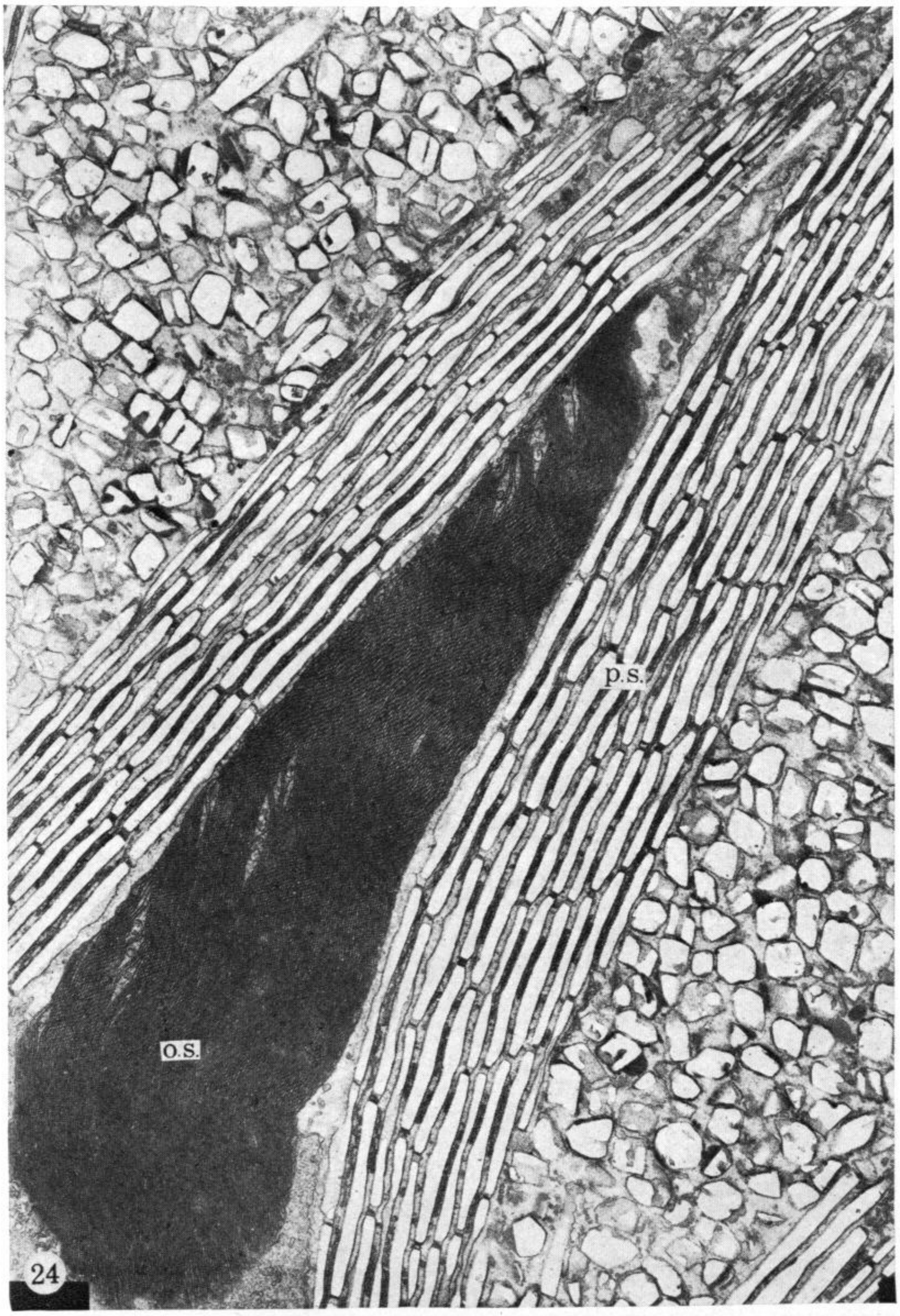



FIGURES 20–21. For description see page 36.




FIGURES 22–23. For description see page 37.

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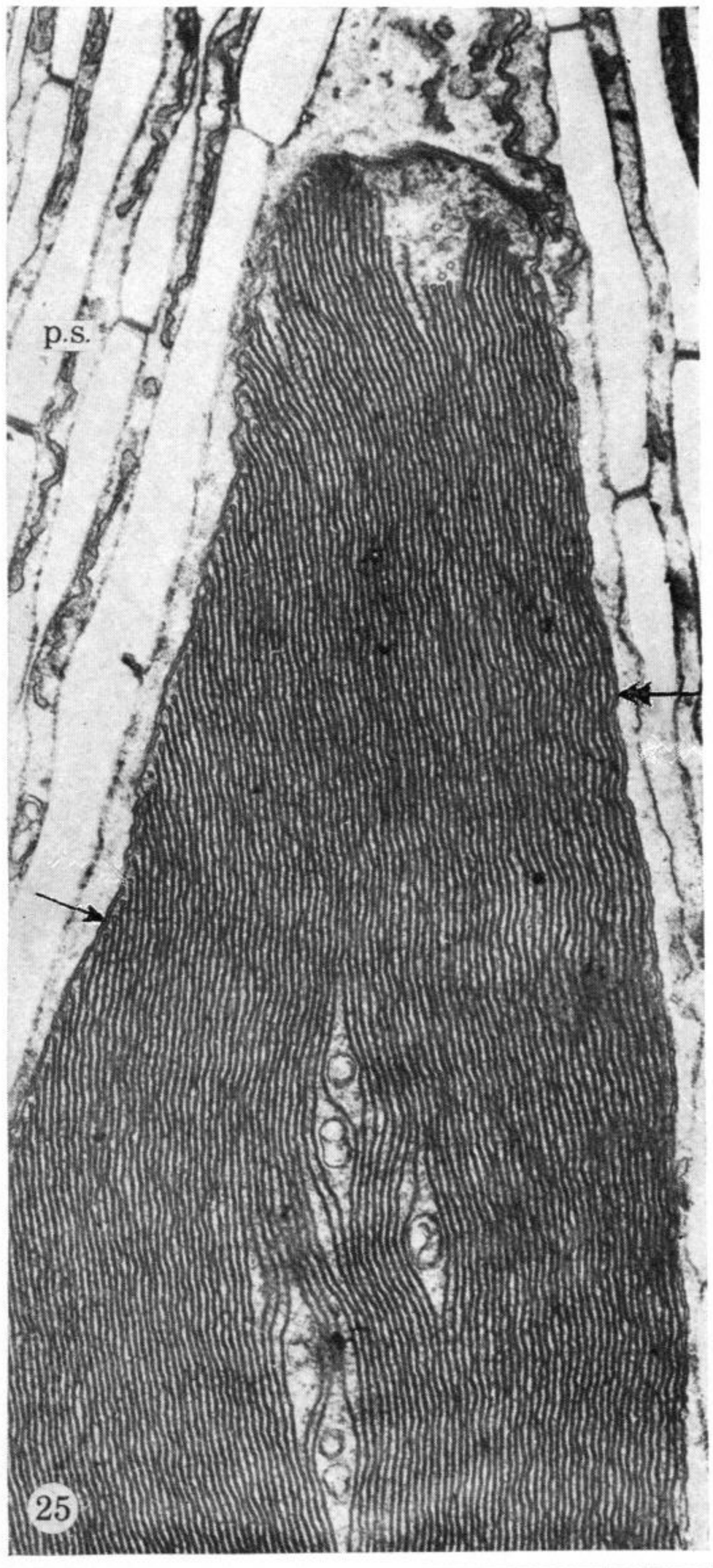


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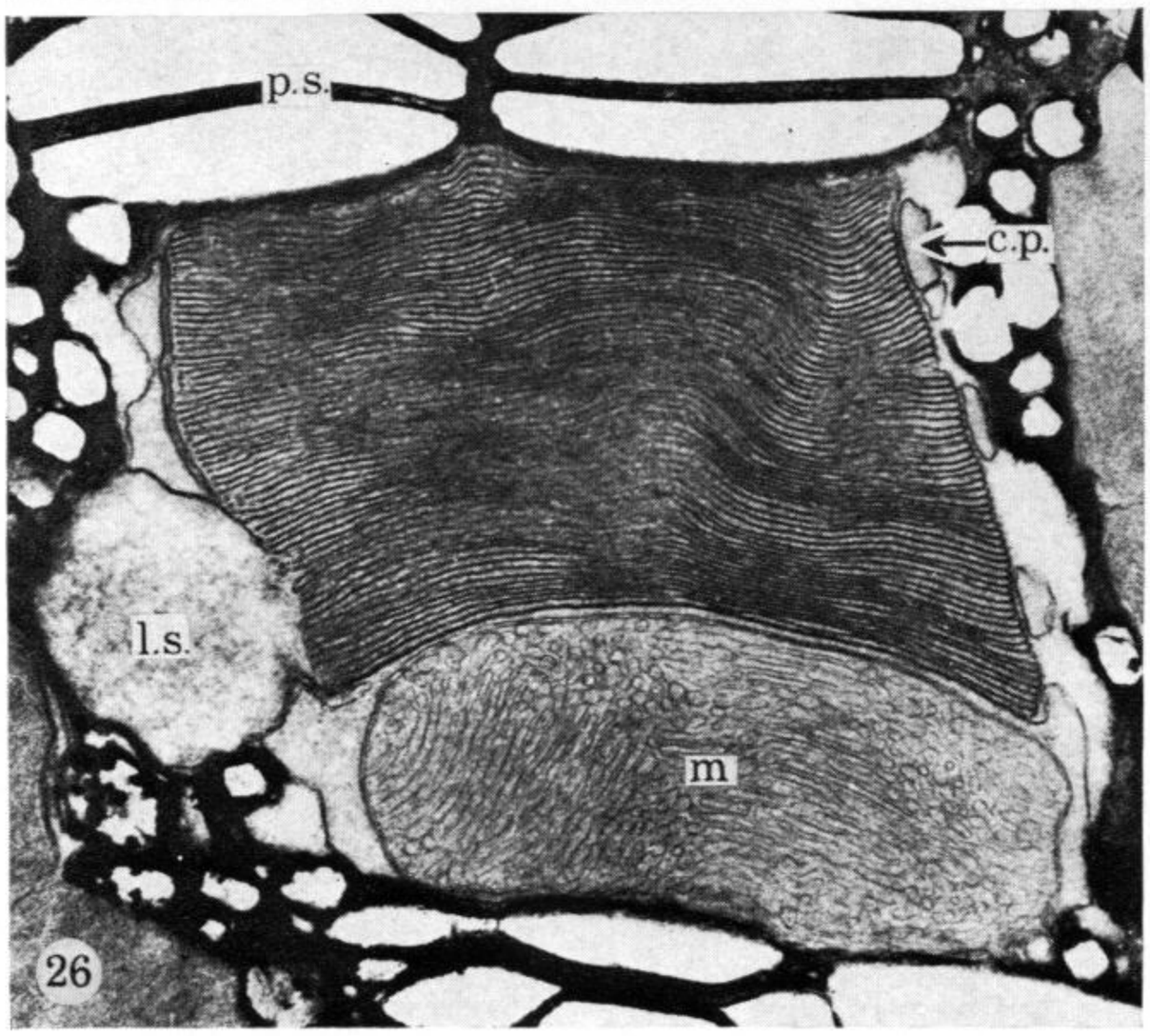


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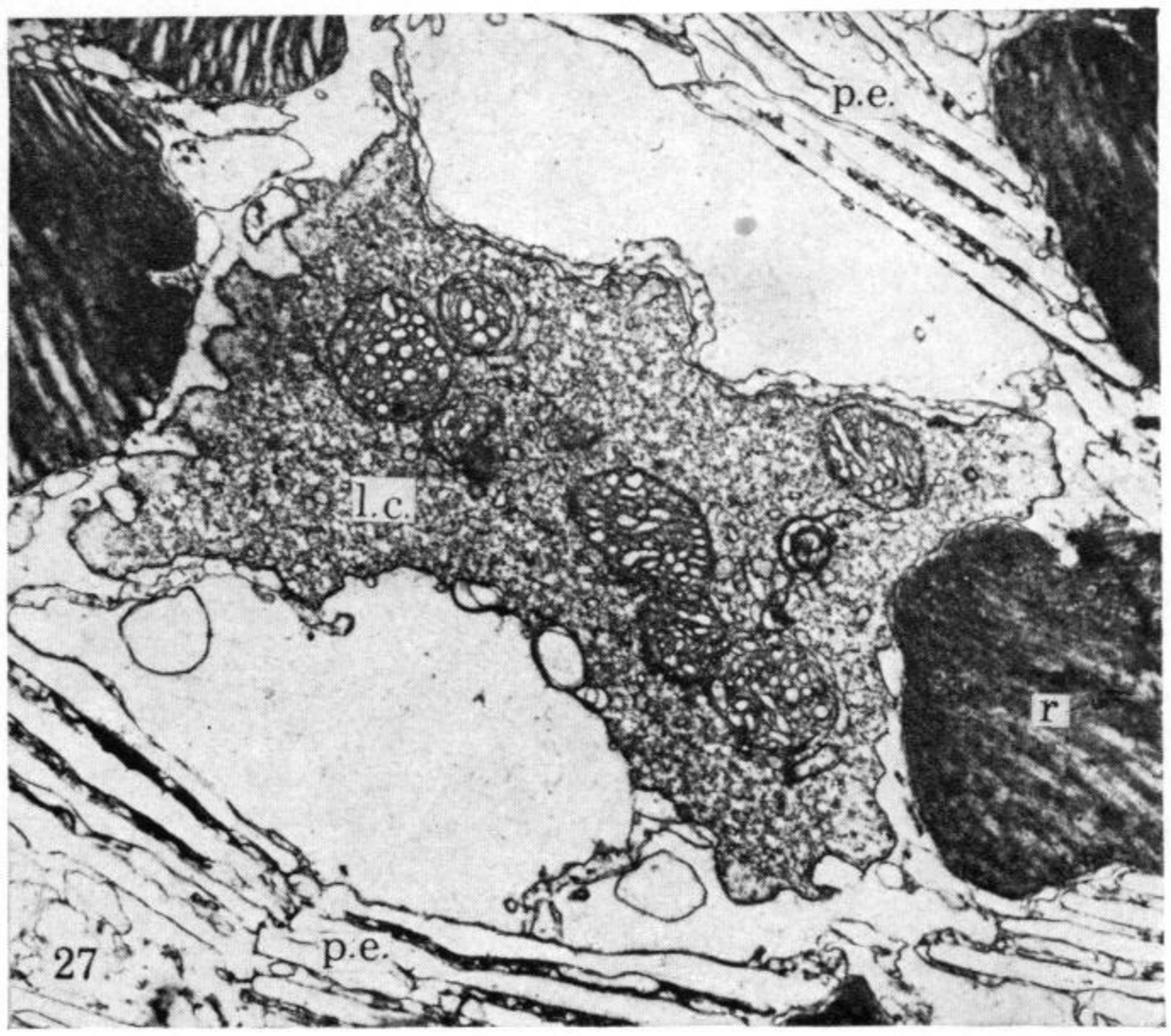


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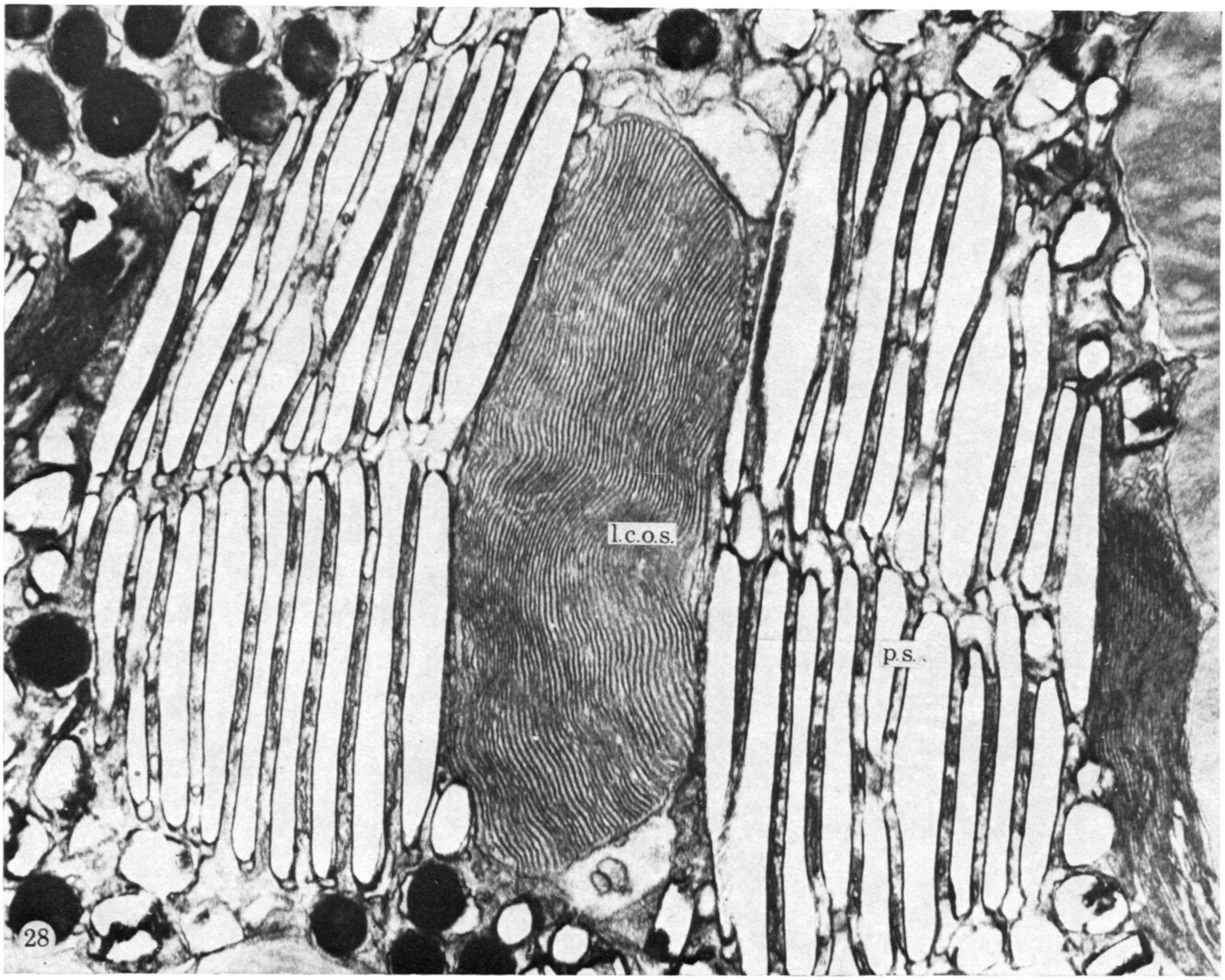


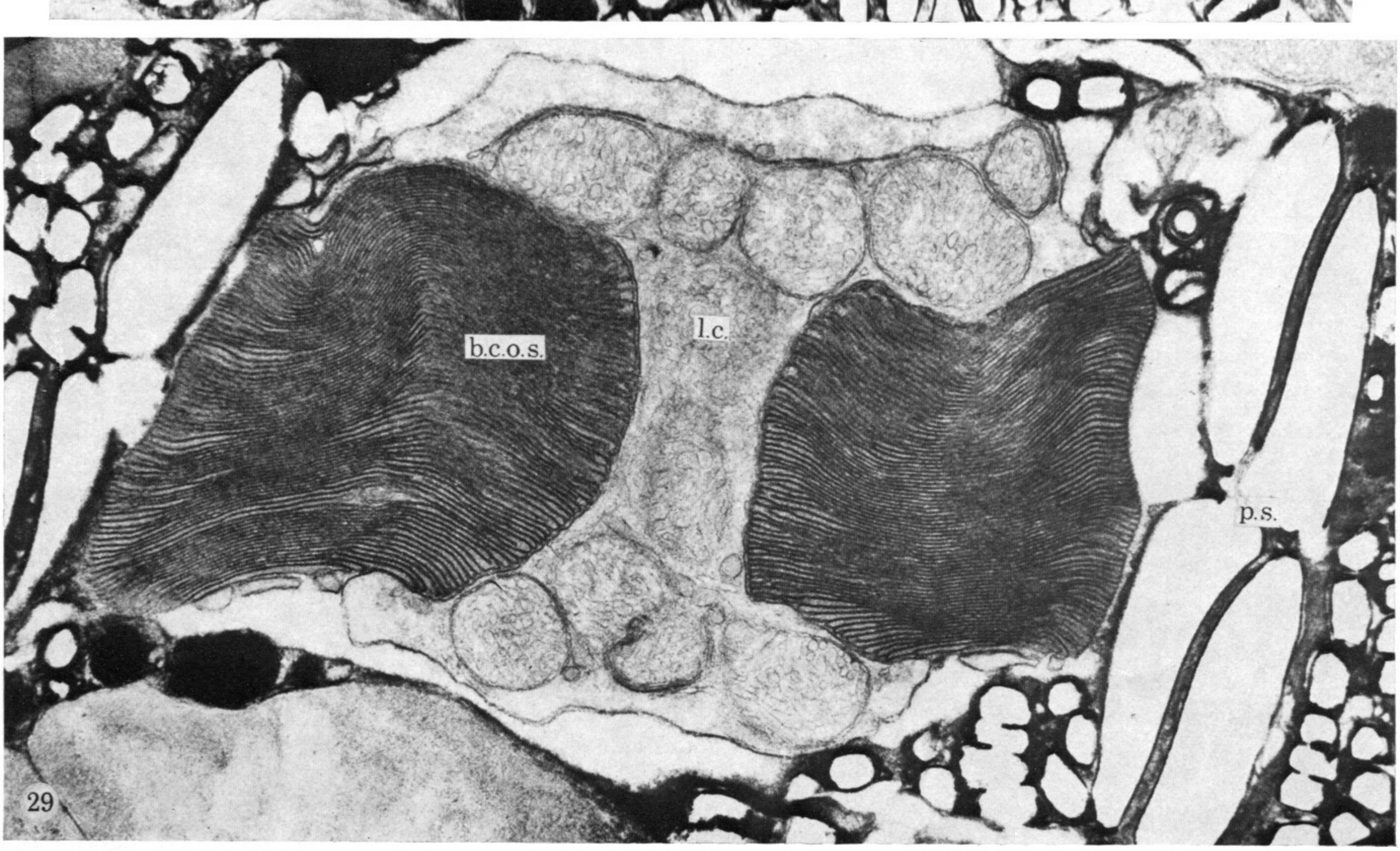


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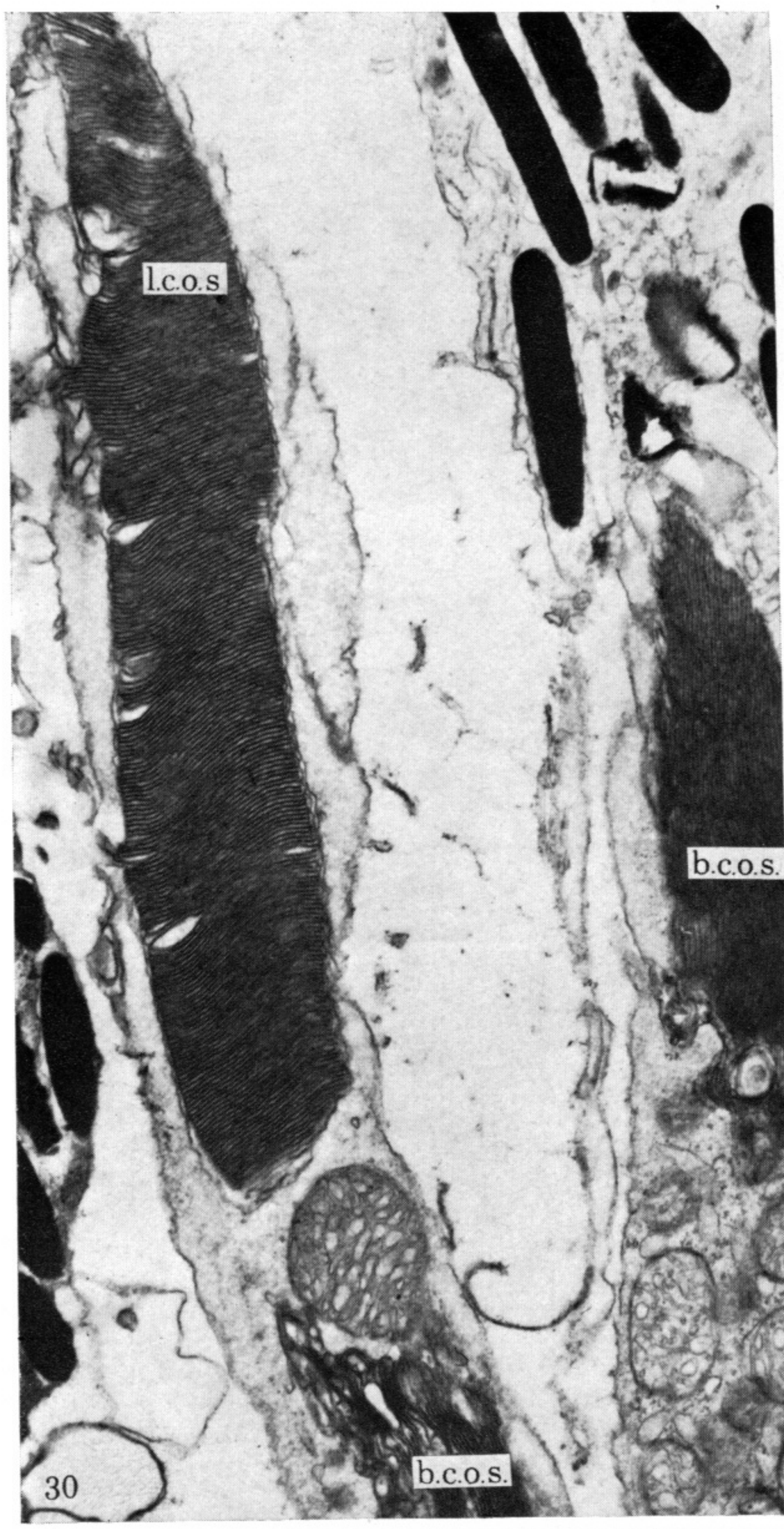
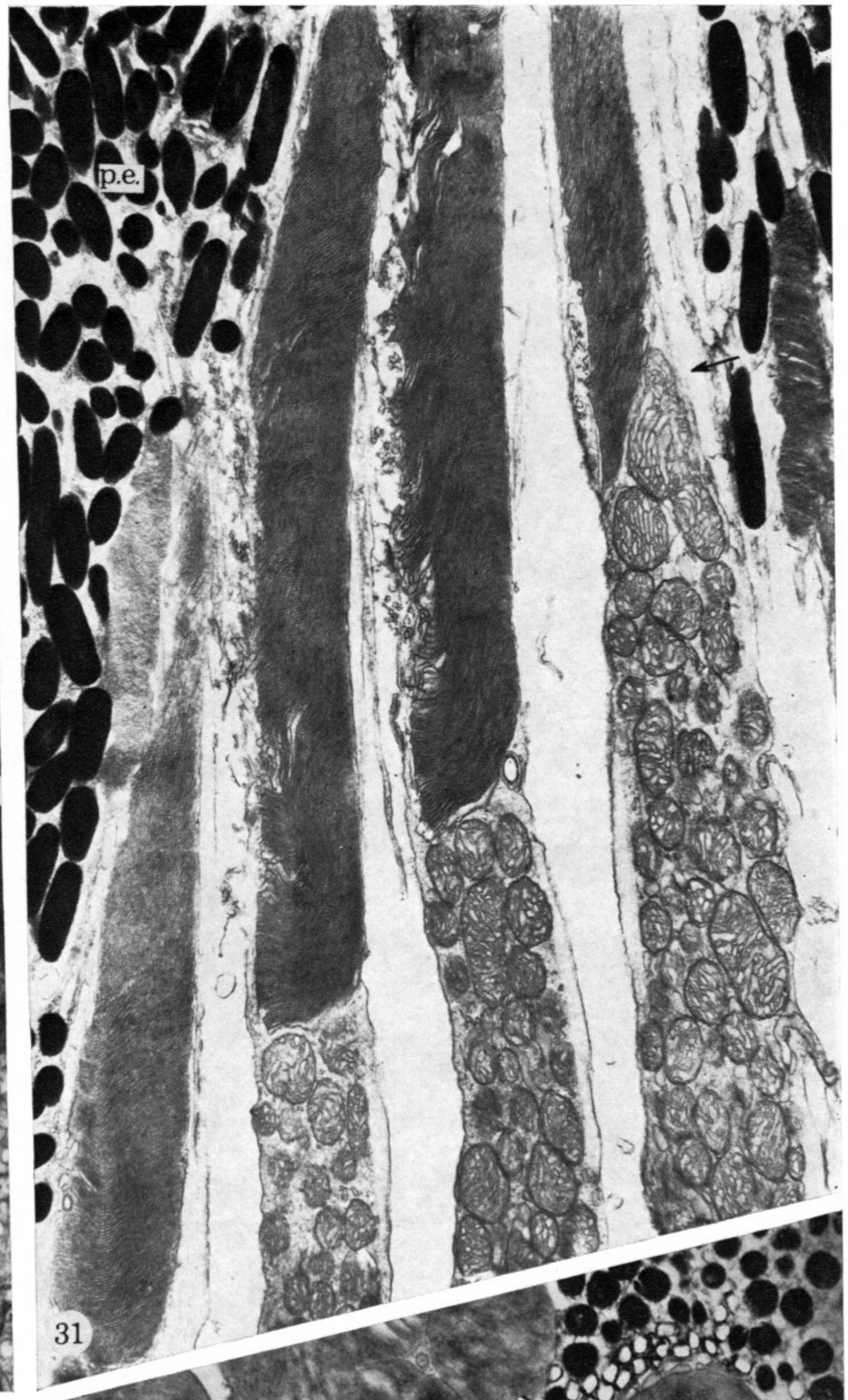
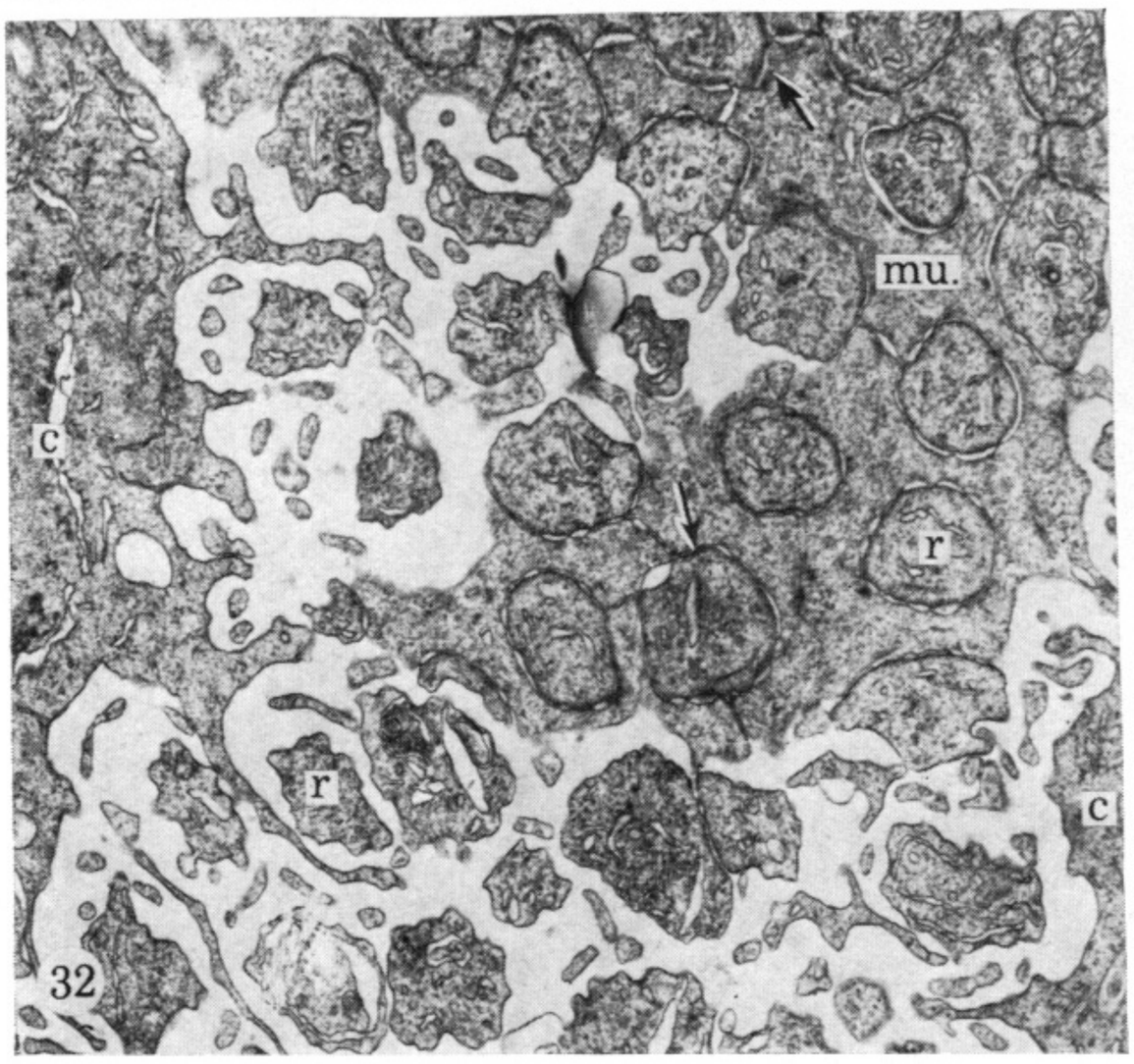
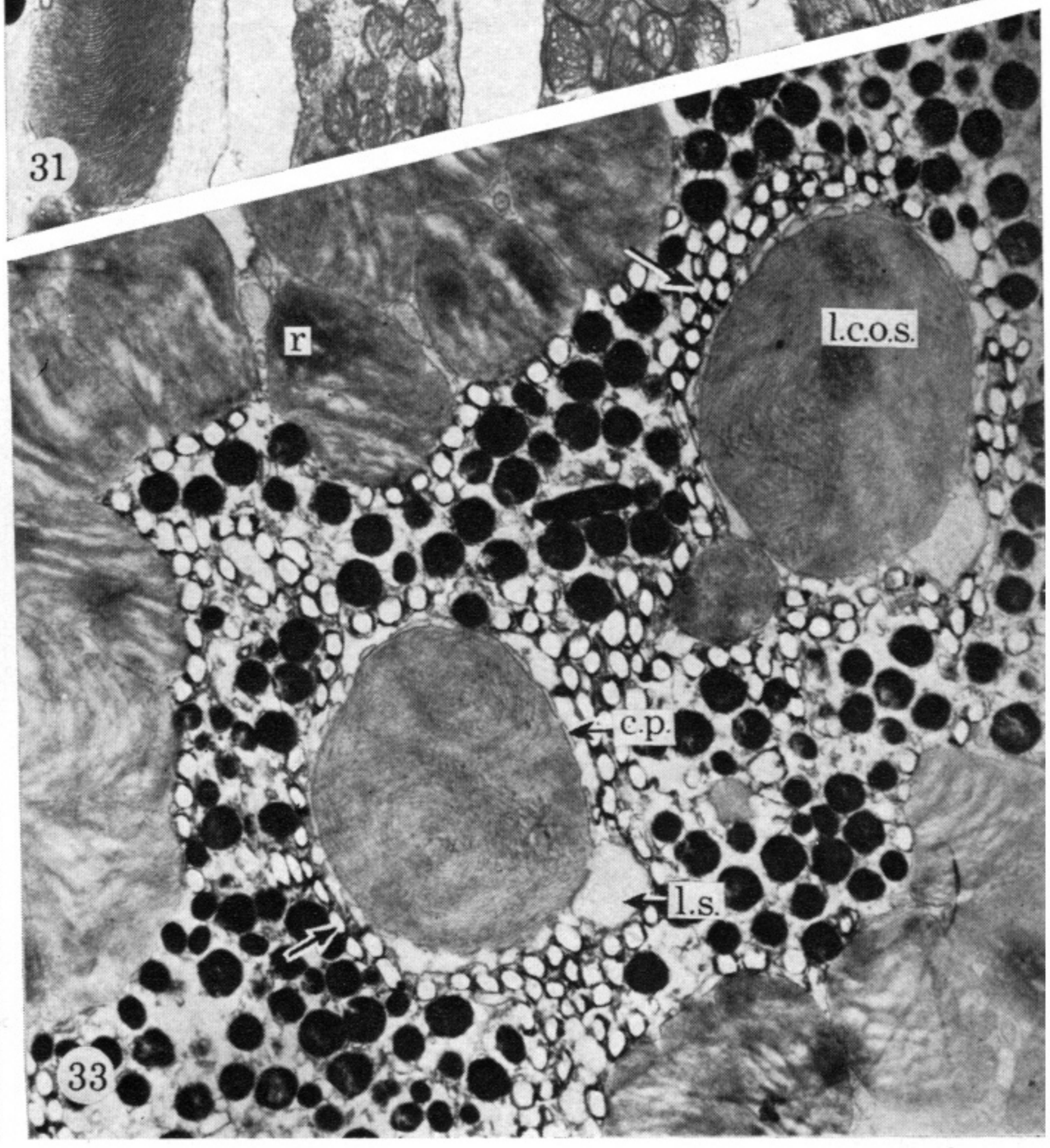
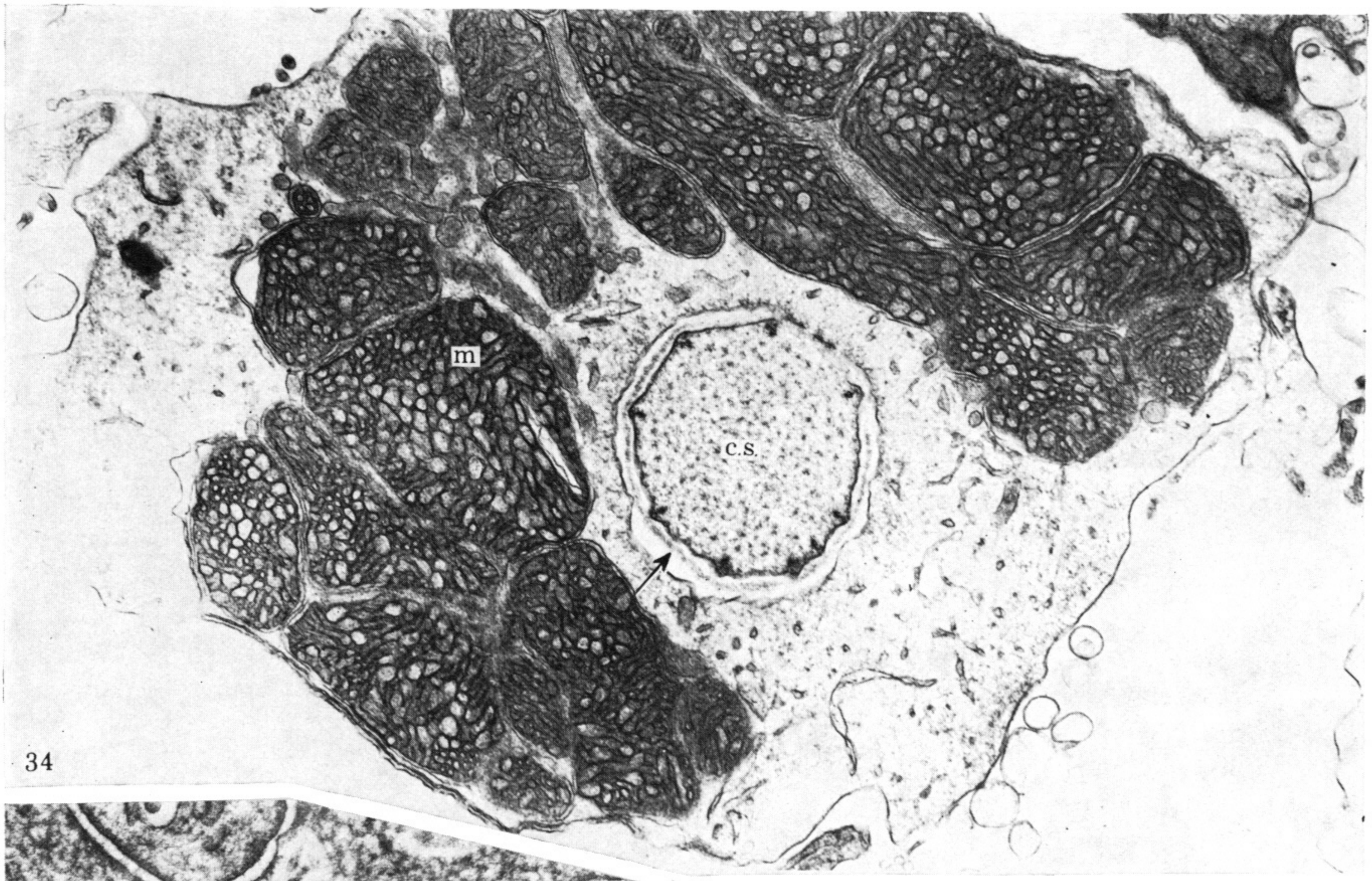



FIGURES 30–33. For description see page 38.

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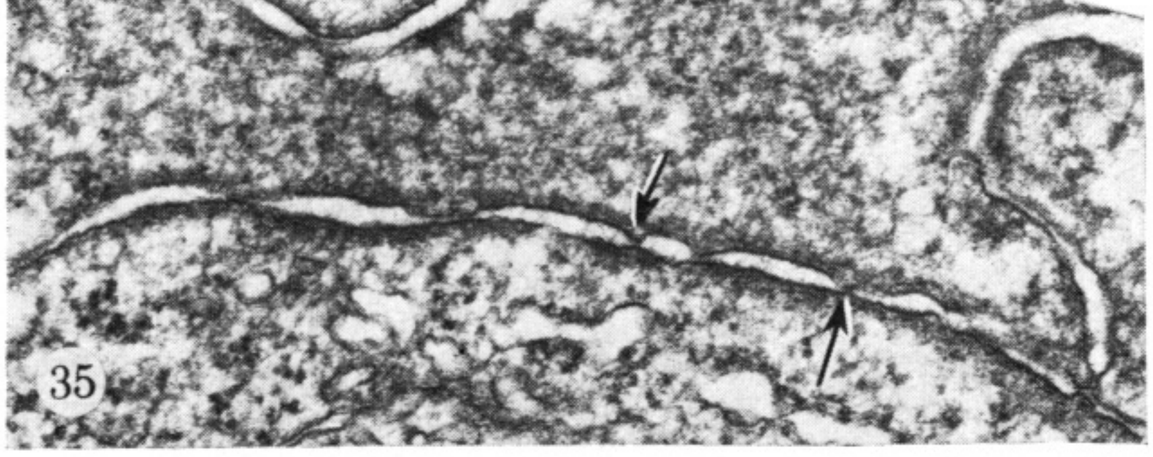

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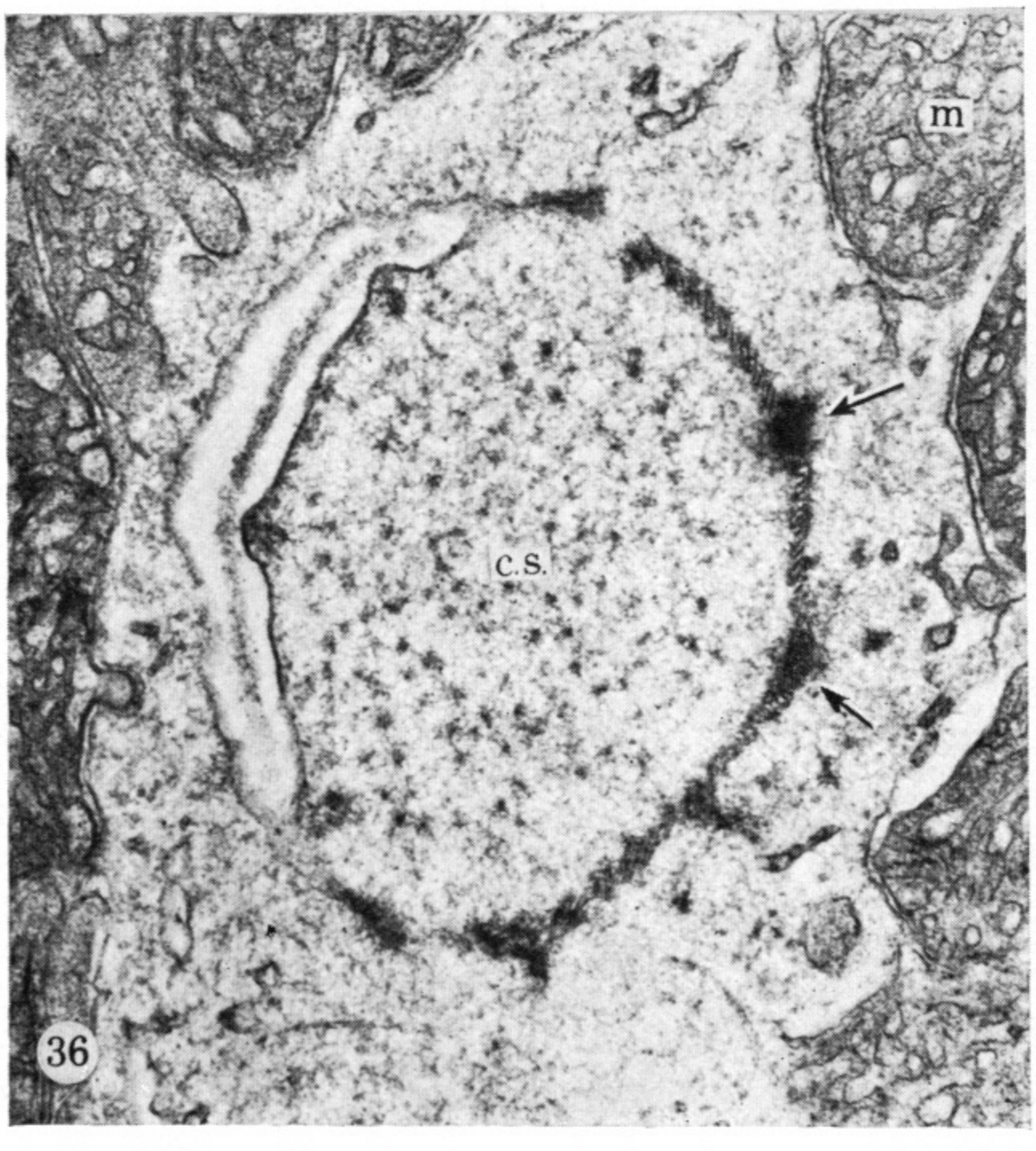


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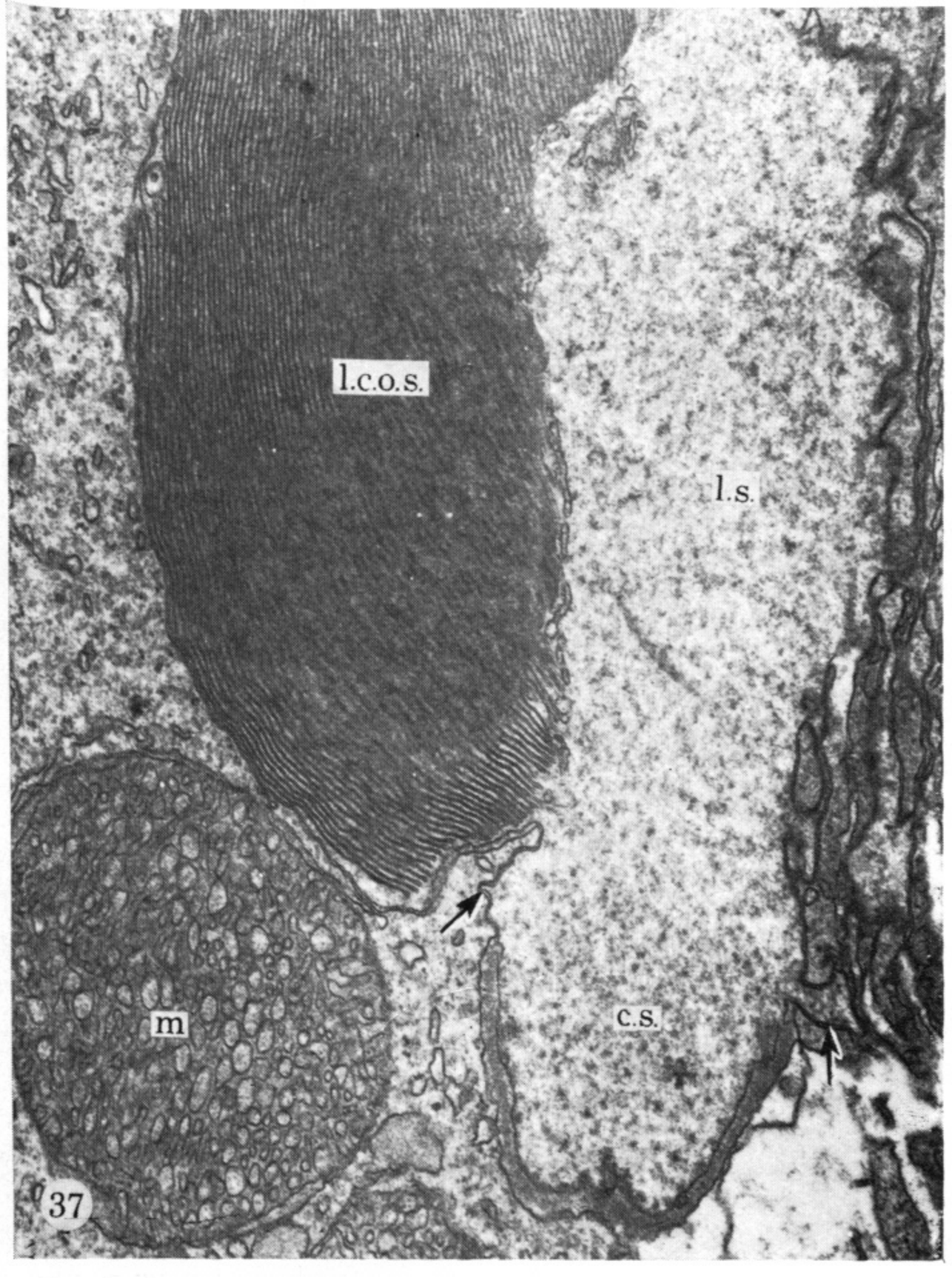


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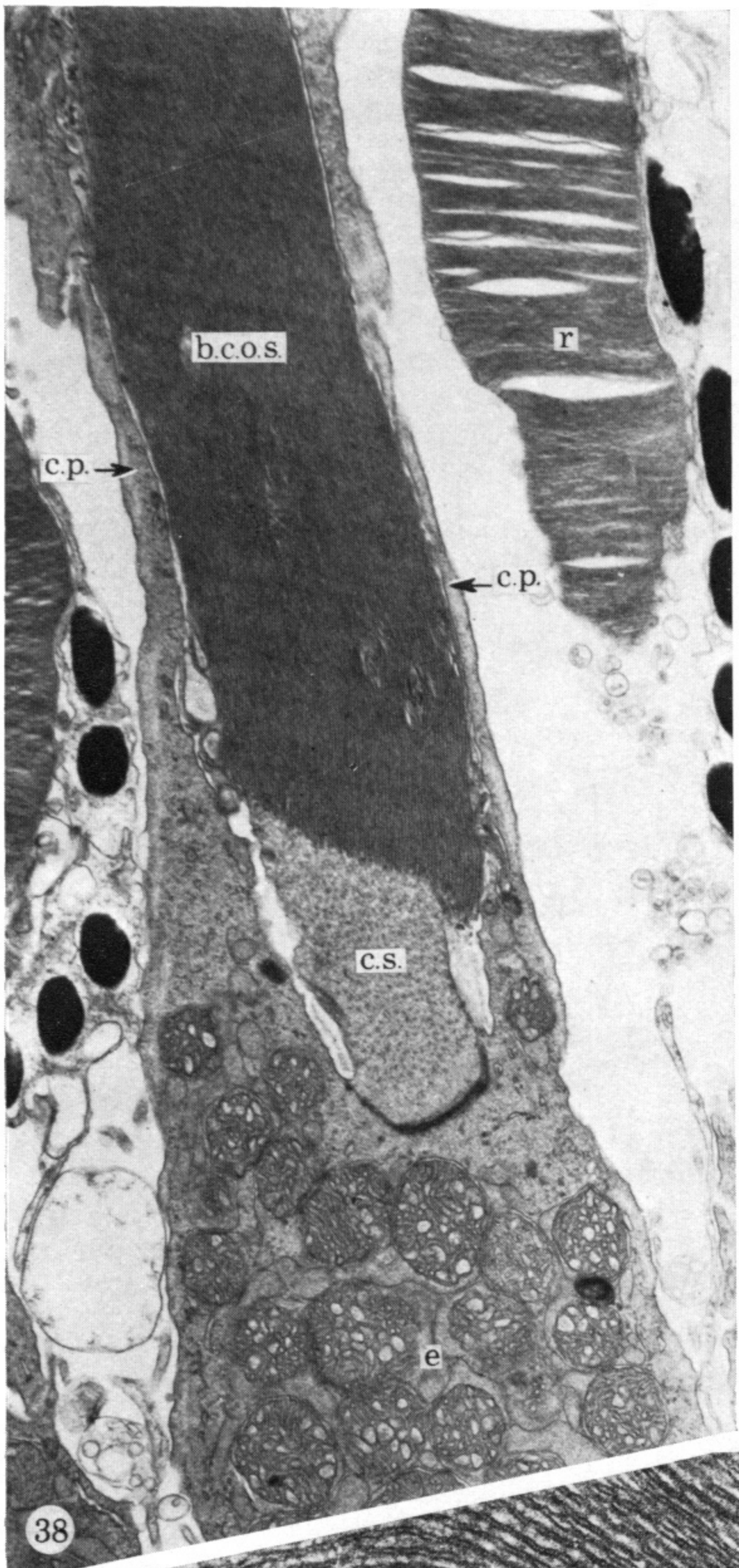


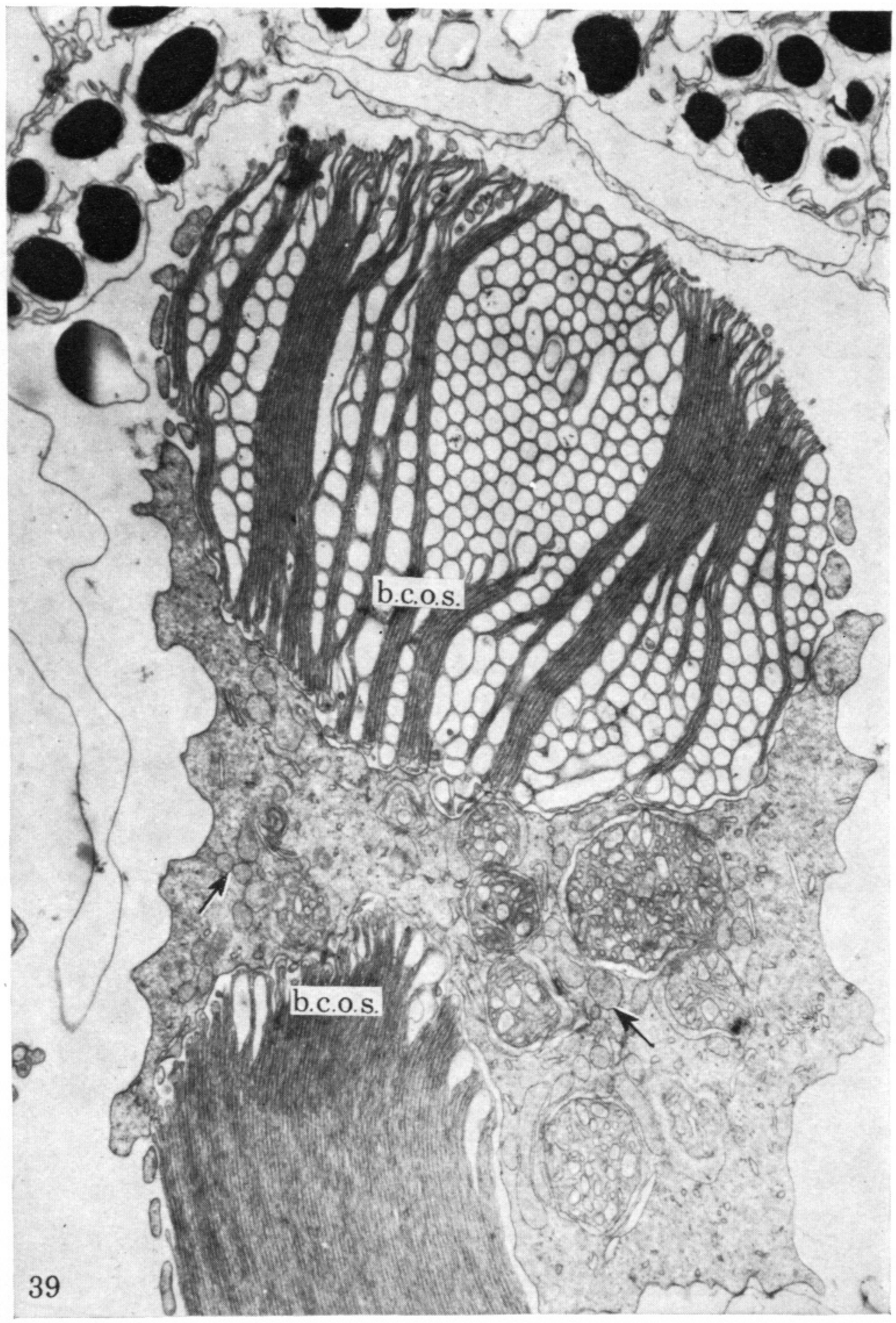


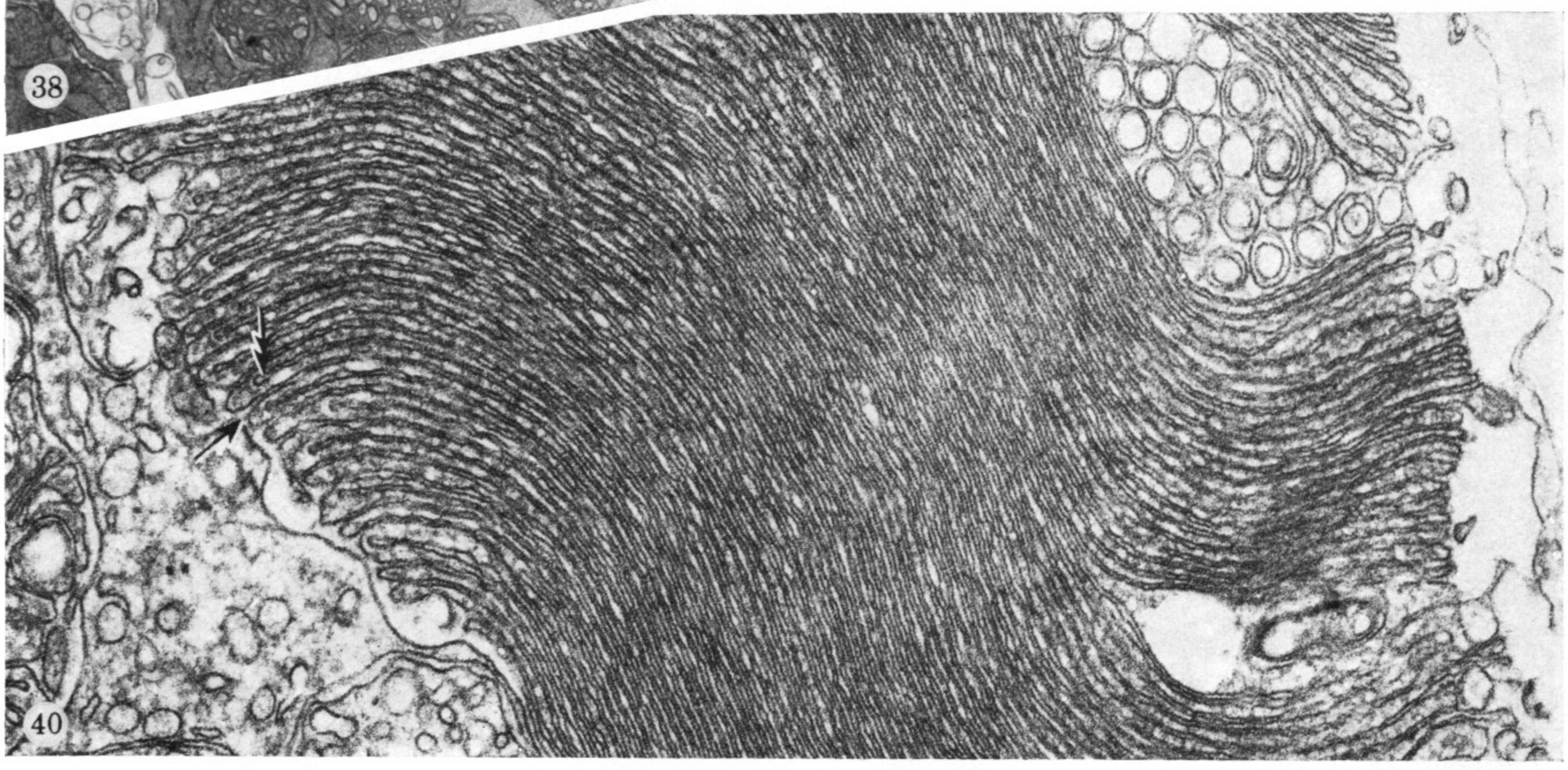


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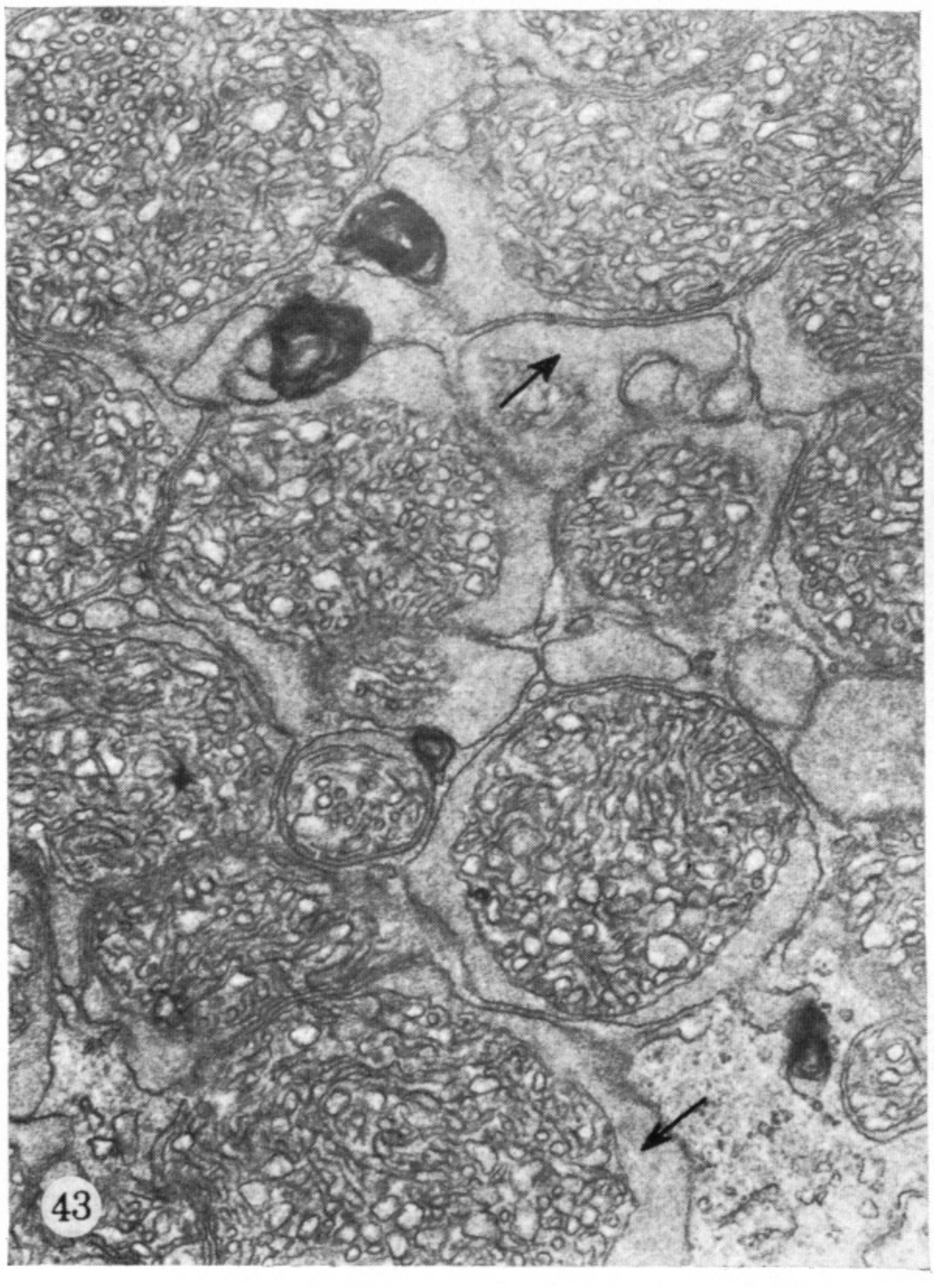


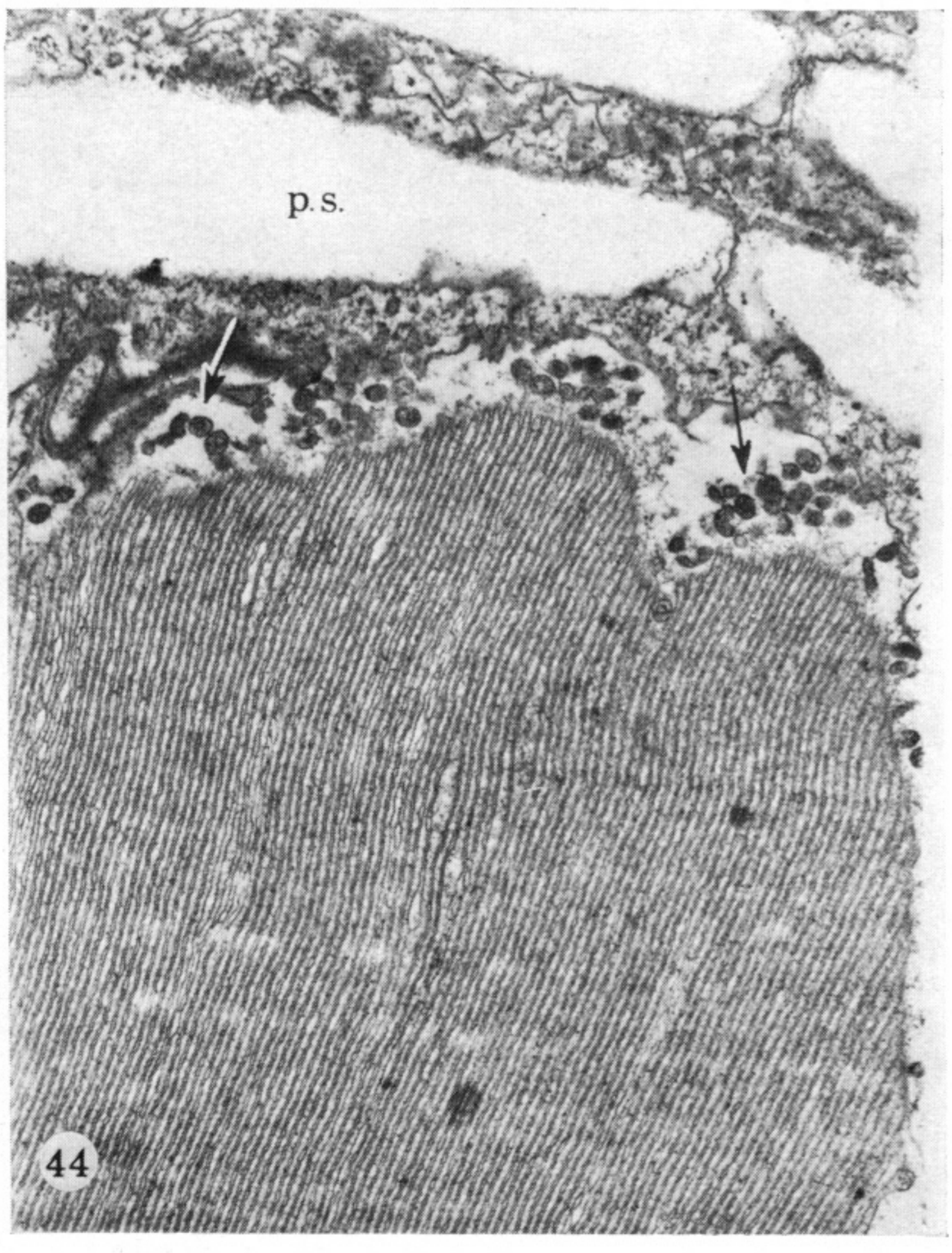


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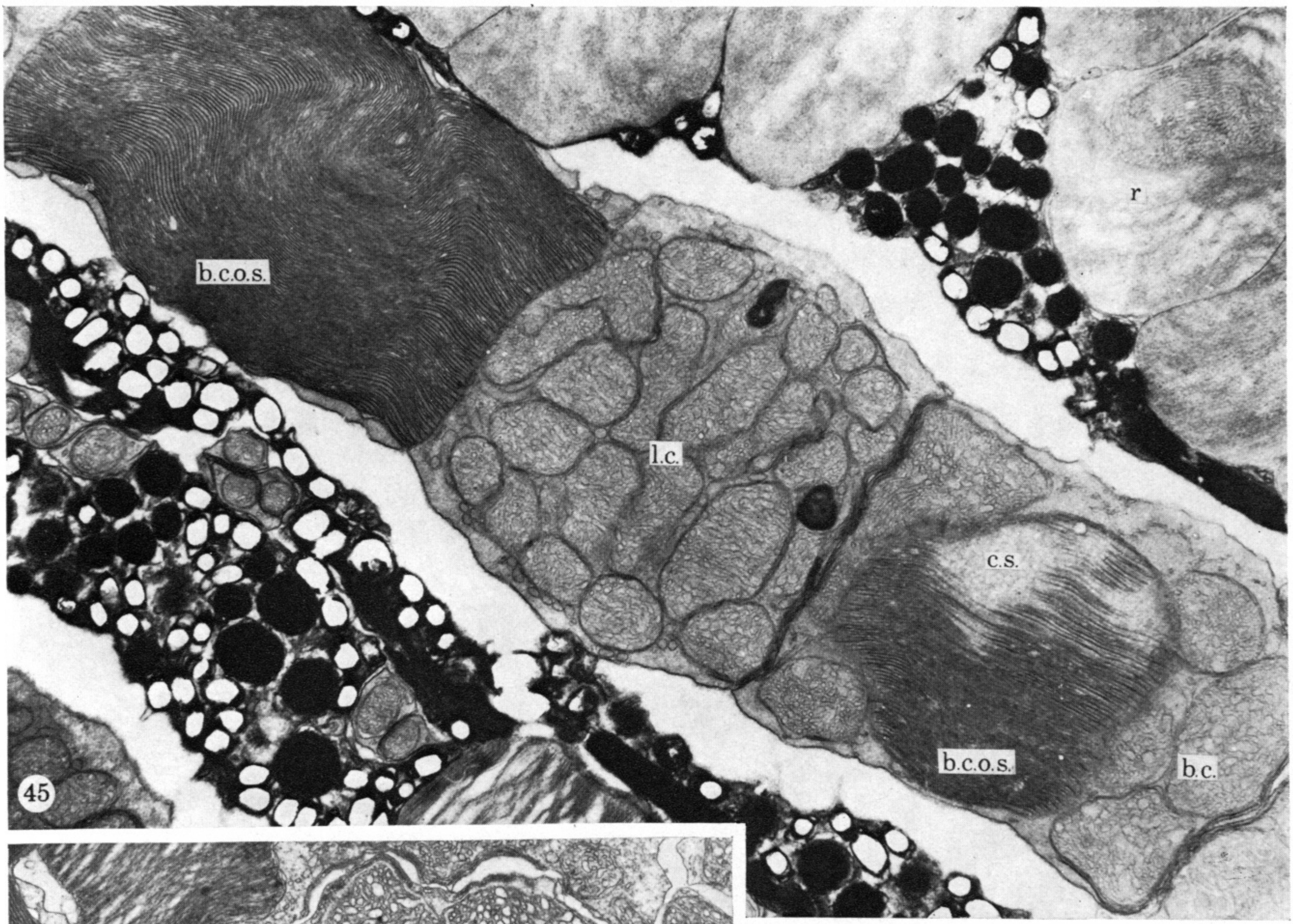
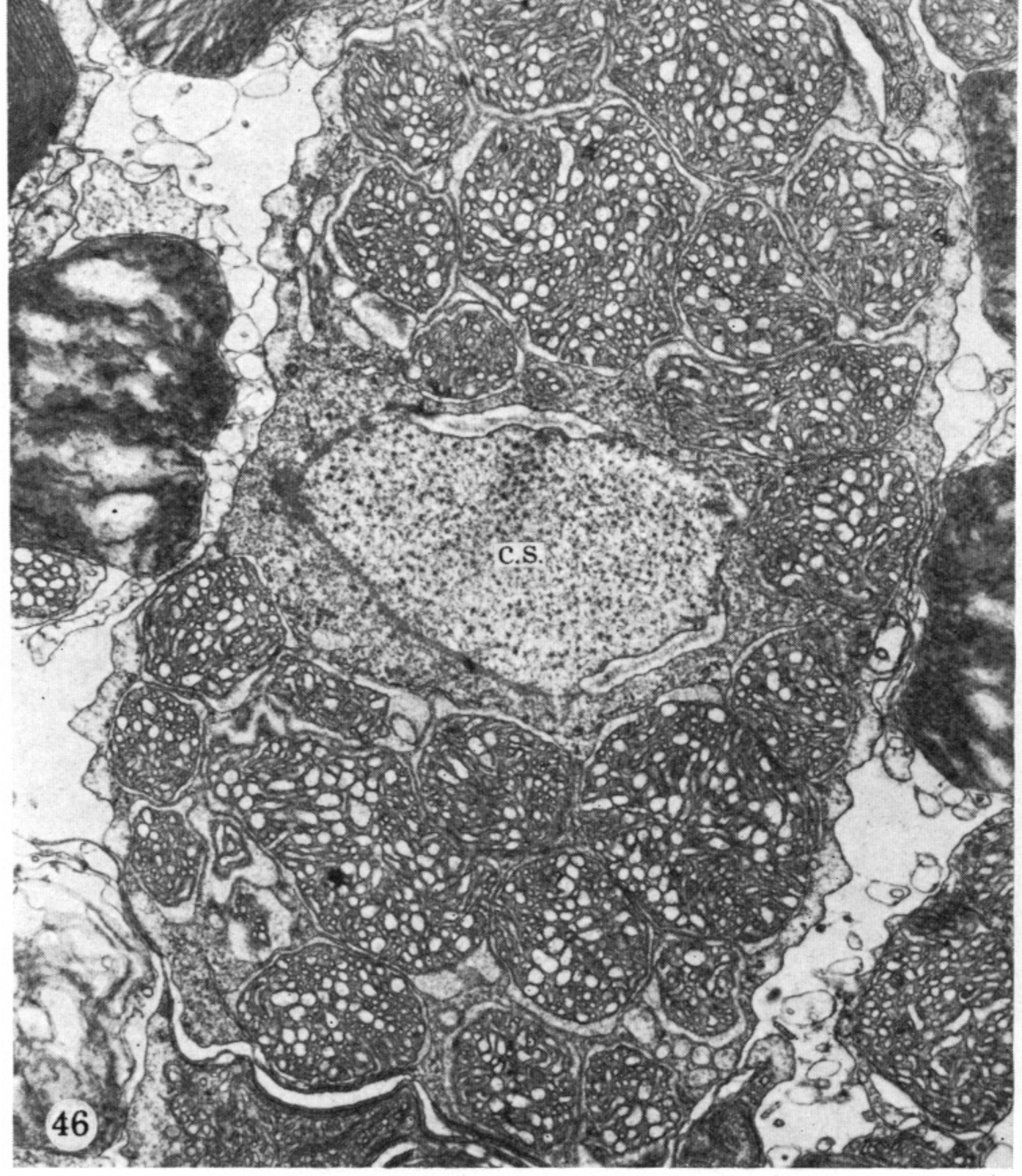
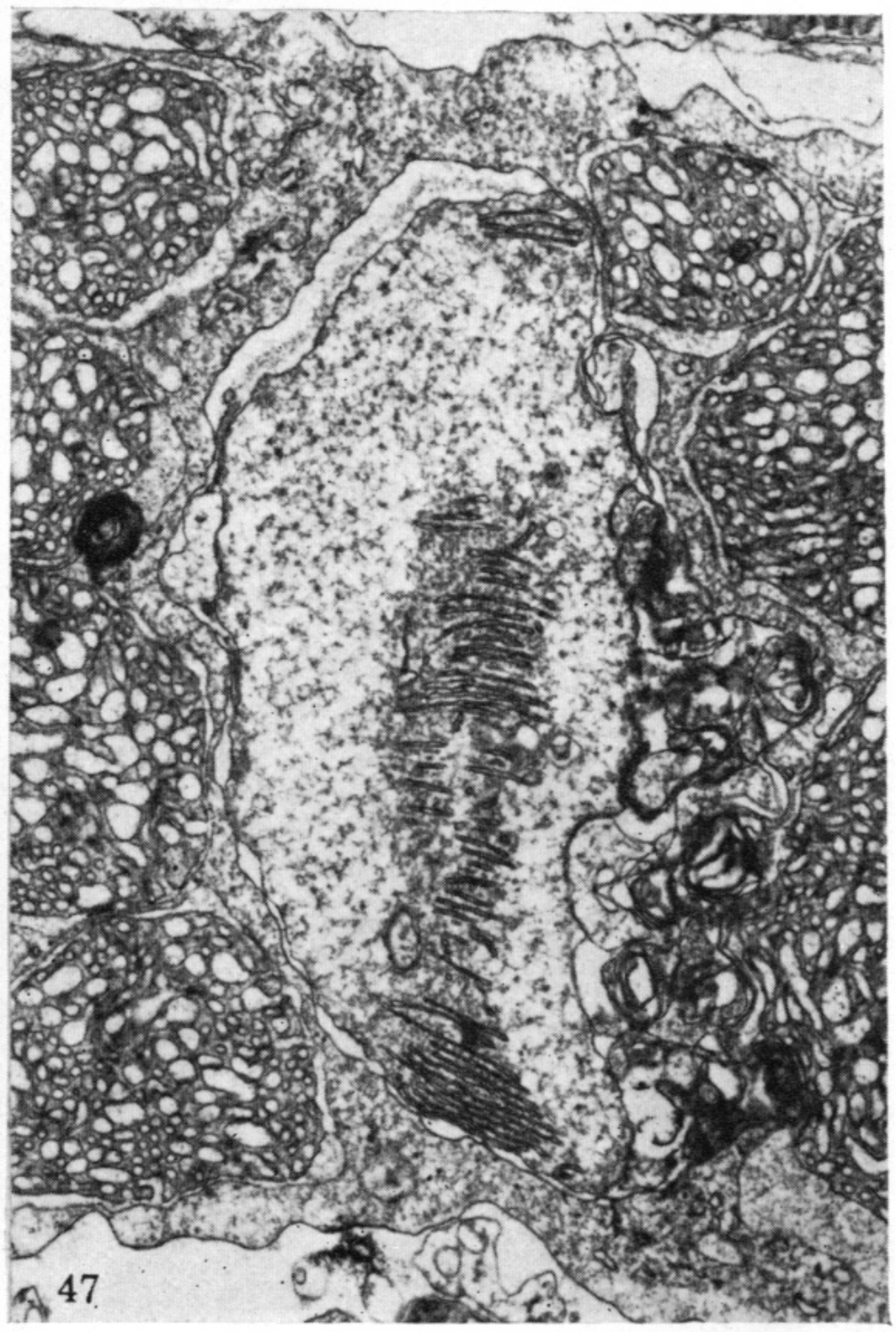
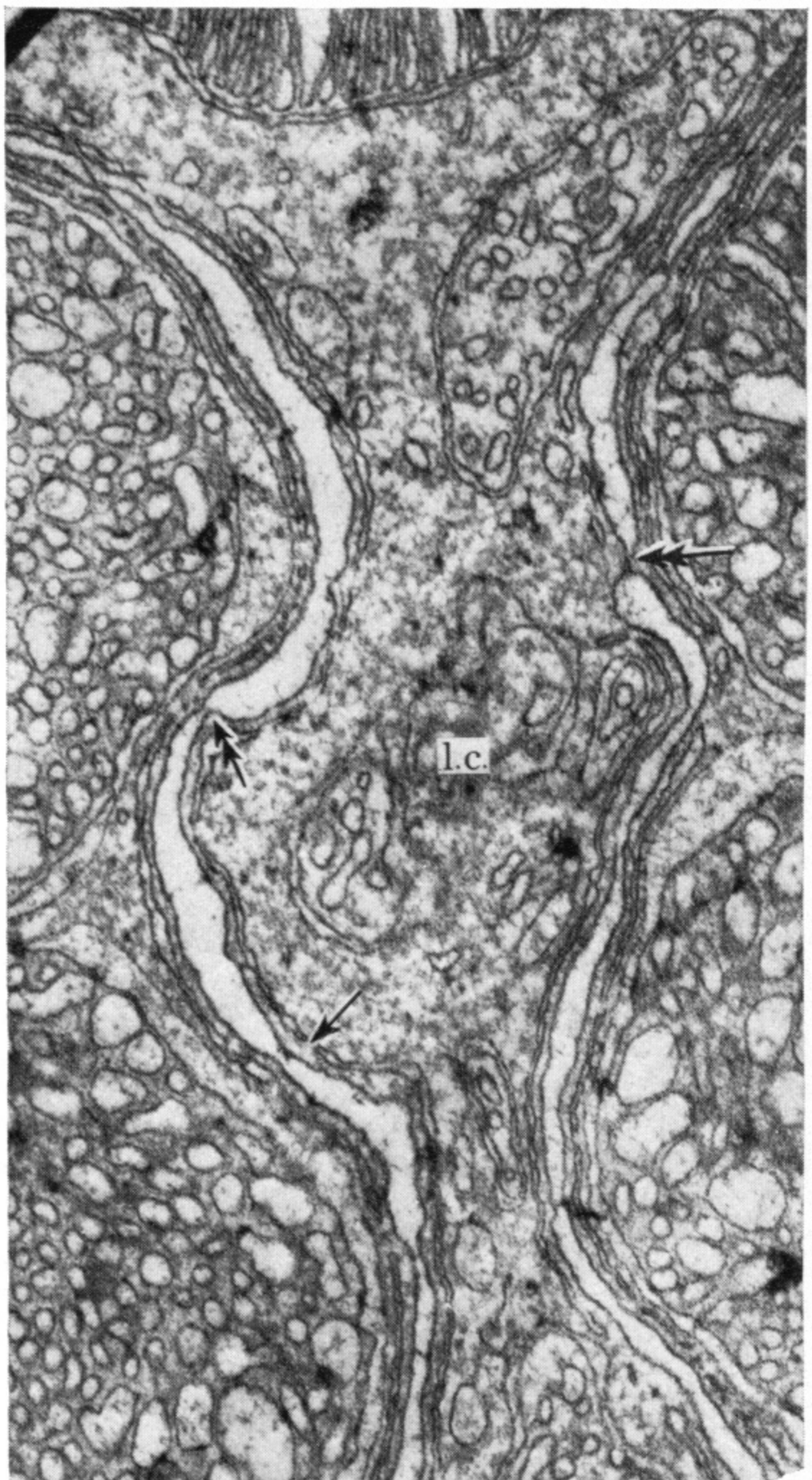



FIGURES 45–47. For description see page 42.


FIGURE 48. For description see page 43.

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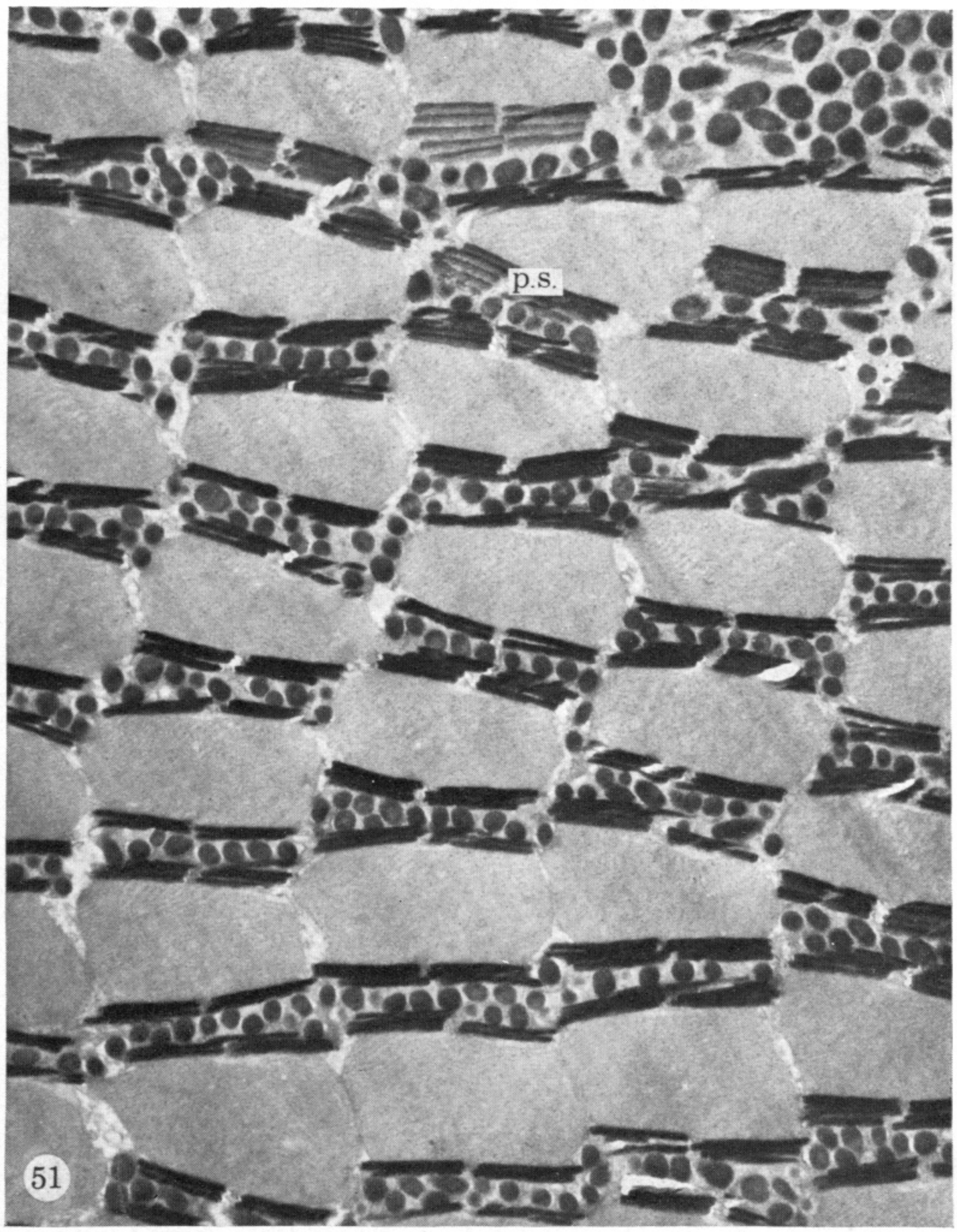
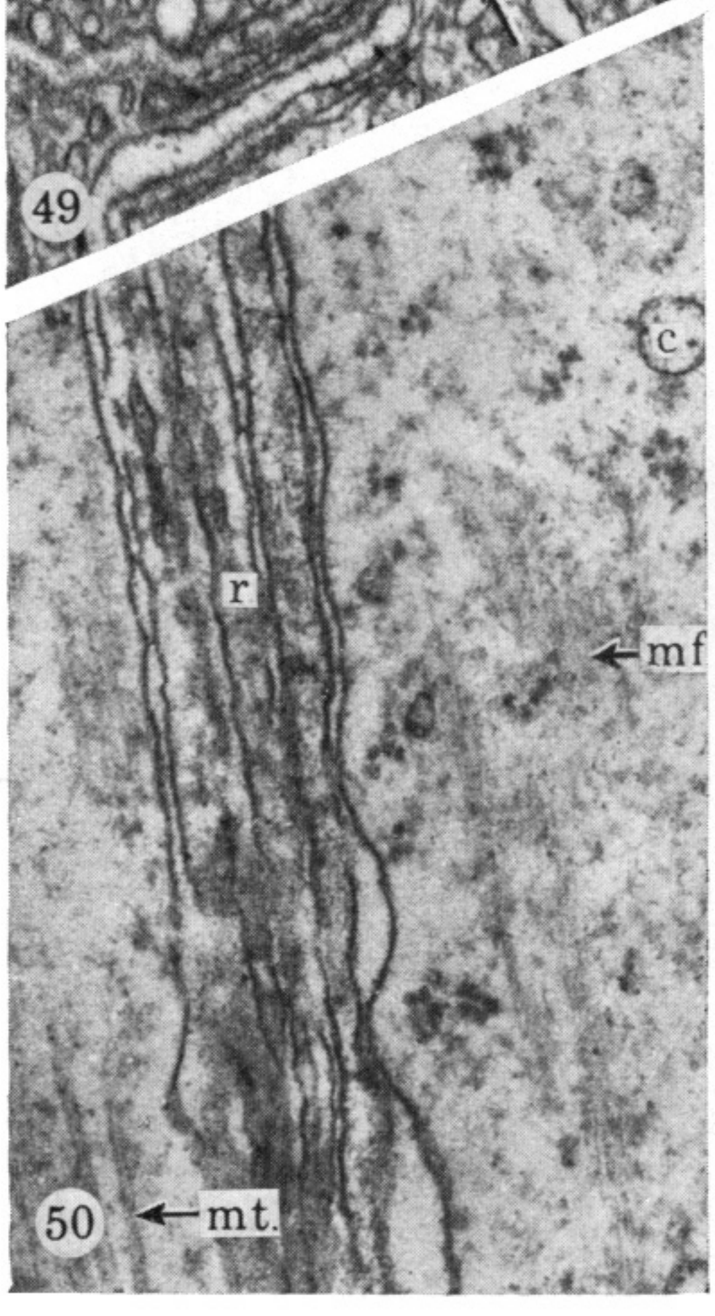
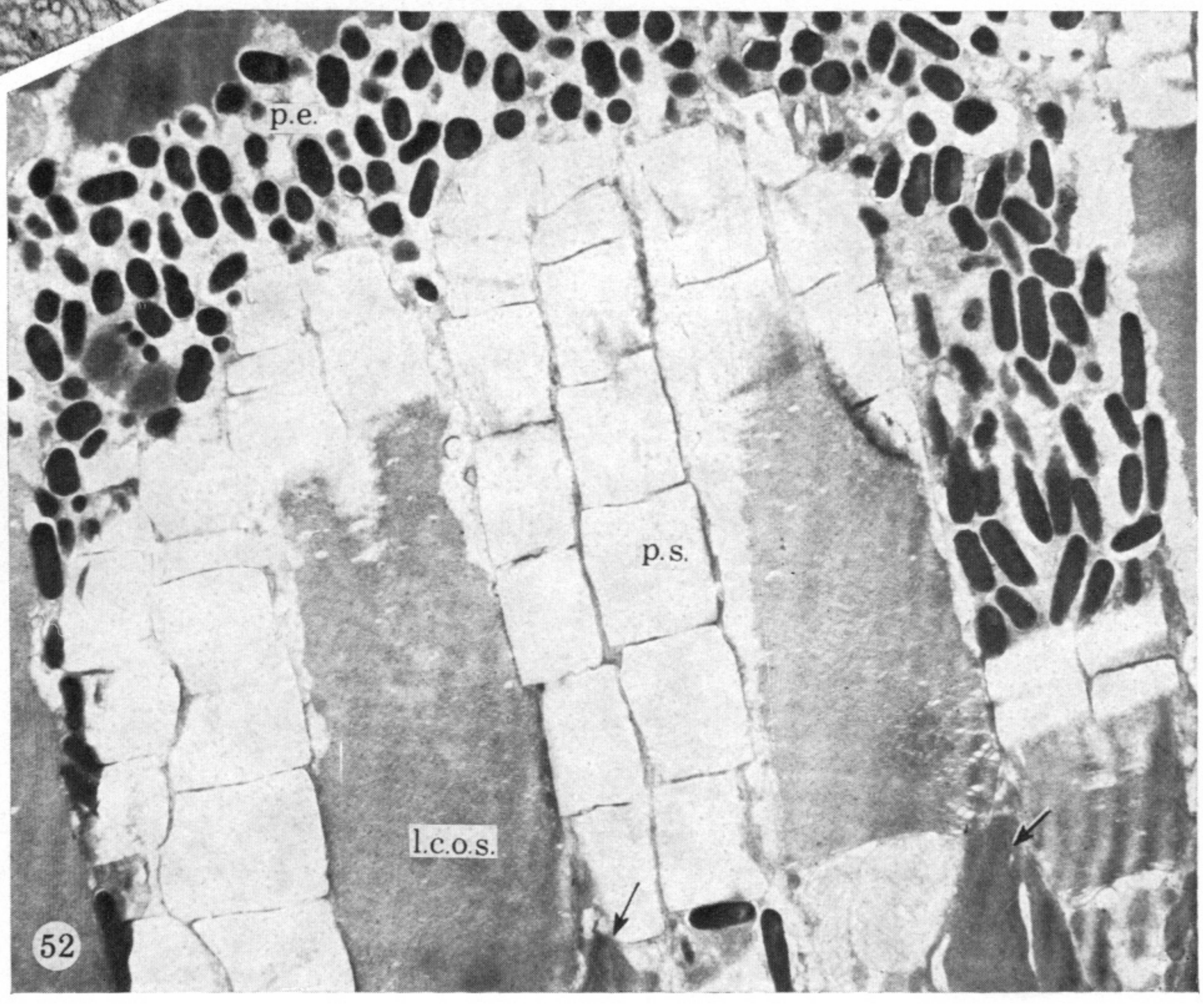



51




50




52

FIGURES 49–52. For description see opposite.