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POST-EMBRYONIC DEVELOPMENT OF THE PRINCIPAL RETINA OF A JUMPING SPIDER

II. THE ACQUISITION AND REORGANIZATION OF RHABDOMERES AND GROWTH OF THE GLIAL MATRIX

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An accompanying paper by Blest (*Phil. Trans. R. Soc. Lond.* B **320**, 489 (1988*b*)) describes the conformational changes undergone by principal retinae of a salticid spider during post-embryonic development, and identifies the origins of the tiered receptor mosaics. We now discuss the ultrastructural morphogenesis of the individual receptive segments, with particular reference to the growth of microvilli, to the achievement of light-guide properties by rhabdomeres of the foveal mosaic of Layer I, and to putative mechanisms that ensure that inter-receptor spacings are optically apt.

1. Establishment of the tiered retina by conformational changes precedes the first appearance of plasmalemmal microvilli in all four Layers of receptive segments. Microvilli are first differentiated in the ventral retina.
2. Short, irregular microvilli, initially interdigitated, appear on the plasmalemmae of receptive segments around day 10 of post-embryonic development. They progressively lengthen, cease to be inter-digitated, and become more regular throughout subsequent development, during the moult to the second instar at day 12, and until day 15 when spiderlings become fully independent and principal retinae attain their final conformation.
3. Until late in development, after the moult to the second instar, foveal Layer I receptive segments are contiguous, and densely occupied by organelles. The six glial strands that flank each receptive segment are prominent. A transformation between days 14 and 15 variously reduces or eliminates the glial columns, and, by endocytosis, the plasmalemmal areas of the receptive segments. The Layer I cytoplasm is stripped of organelles other than microtubules. Consequently, receptive segments become separated from each other, and their rhabdomeres can perform as light-guides.
4. Over the same period, receptive segments of Layers II–IV slowly acquire the dense population of mitochondria that have been shown to equilibrate the refractive indices of rhabdomeres and their surrounds.
5. Final conformational changes to the retinae and the establishment of definitive spacings between receptive segments seem to be assured by the differential growth and local involution of the finely divided glial processes intercalated between them.

Despite some caveats, it is argued that this protracted sequence of events is consistent with a limited degree of ontogenetic recapitulation. In particular, it is remarkable that the early states of the nascent microvilli so closely resemble those found in less advanced families of spiders.

1. Introduction

An accompanying paper (Blest 1988*b*) describes the gross conformational changes undergone by the principal retina of a jumping spider, *Plexippus*, during post-embryonic development and the first three days of the second instar. The generation of four tiers of receptive segments was shown largely to result from changes to the conformation of the whole retina. The special properties of each of the four tiers (Layers) relate to their individual ultrastructures (Blest & Price 1984; Blest & Sigmund 1984; Blest & Sigmund 1985*a*).

1. Rhabdomeres of Layer I, farthest from the dioptrics, are organized as long light-guides (Williams & McIntyre 1980; Blest *et al.* 1981; Blest 1985*b*; Blest *et al.* 1988).
2. Receptive segments belonging to Layers II and III at the fovea present mosaics composed of more-or-less confluent rhabdomeres. Their cytoplasm is densely occupied by mitochondria that equilibrate the rhabdomeral and cytoplasmic refractive indices, and may have no other function (Williams & McIntyre 1980; Blest *et al.* 1981).
3. Layer IV receptors at the outer side of the retina (4a receptive segments) present a

dorsoventral linear arrangement, each segment bearing two rhabdomeres with orderly arrangements of microvilli. Eakin & Brandenburger (1971) noted that they should be able to sustain polarization analysis, which in terms of all attempts at optical modelling (Land 1969; Williams & McIntyre 1980; Blest *et al.* 1981) would be conducted in the UV.

Whatever the functional significance of these highly ordered ultrastructural dispositions, one may ask: how are they acquired during morphogenesis? Assuming the element of ontogenetic recapitulation discussed in the accompanying paper (Blest 1988*b*), can they tell us anything about how such functionally sophisticated mosaics evolved?

We describe the post-embryonic morphogenesis of receptive segments in all four Layers of the tiered principal retina of *Plexippus*, but with particular reference to the differentiation of light-guiding architecture by foveal Layer I.

2. Materials and methods

We used the same sample of developing retinae that are discussed at the level of light microscopy by Blest (1988*b*). Thin, silver to pale gold sections of retinae embedded in Araldite were stained with uranyl acetate and lead citrate and examined under a Hitachi H-600 electron microscope.

The reservations expressed by Blest (1988*b*) about the sometimes ambiguous orientations of horizontal or sagittal sections of principal retinae apply equally to an ultrastructural analysis. This account deals largely with transverse sections. Care was taken to ensure that sections were as strictly transverse as possible, using criteria from larger fields of view than those illustrated in the plates.

The notation for days of development follows the convention explained in detail in the accompanying paper (Blest 1988*b*).

3. Results

3.1. *The ultrastructure of nascent receptive segments from days* 3–8′

At day 3, primordial receptive segments are untiered and shallow. Despite their recognizable organization in light micrographs as a 'honey-comb' in transverse section, (*a*) their plasmalemmae are not equipped with microvilli, and (*b*) the six longitudinal glial strands that flank each receptive segment are slender (figures 1 and 2, plate 1).

At day 7′, microvilli are still absent, but the future organization of the tiered retina is apparent. Figure 3, plate 2, shows a transverse section of a retina at day 7′, taken just proximal to the future 'pit'. The ultimate identities of the nascent receptive segments that at this stage lie at the same transverse plane can be identified by reference to the light micrographs of transverse sections of a principal retina of *Plexippus* published by Blest *et al.* (1981). Eventually, the strip of Layer IV receptive segments identified as 4a will provide the outer, dorsoventral strip of 4a receptive segments; 4b receptive segments are readily identified by their mosaic pattern, in conjunction with their later fate (see below). The dorsoventral strip of receptive segments that lie immediately adjacent to 4a can be predicted to yield 3a receptive segments, and the large field adjacent to it on the inner side, 3b segments. In mature retinae, the 4a and 4b mosaics are contiguous. We infer that primordial Layer III and IV receptive segments undergo a degree of differential axial migration at a later stage of development. The migration is demonstrated below (§3.2).

Figure 4, plate 3, provides a sagittal section of a retina taken near to the fovea at day 7′, and figure 5, plate 4, of presumptive Layer I receptive segments close to the fovea, at higher magnification. Receptive segments contain numerous microtubules and saccular organelles. The glial strands are now well developed, and the retina is bounded distally by an outer limiting membrane. The identities of the receptive segments cannot be reliably established, but a degree of tiering is evident. Figures 6 and 7, plate 5, show transverse sections of receptive segments in Layers III (3a) and IV (4a) (figure 6), and Layer I (figure 7). With the exception of 4a profiles, which are juxtaposed to the glial matrix and are pentagonal, receptive segments are hexagonal and are flanked by six glial strands of substantial widths.

At day 7′, receptive segments around the fovea still do not bear microvilli. Those in the ventral arm, however, possess irregular, interdigitated microvilli reminiscent of the ‘rhabdomeral networks’ of more primitive araneomorph spiders (Blest 1985*a*; Blest & Sigmund 1985*b*). Figures 8–10, plate 6, show transverse sections of receptive segments in the ventral arm of a retina, figure 8 displaying an entire receptive segment, and figures 9 and 10 details of the interfaces between contiguous receptive segments. Figure 8 reveals the wide and irregular nature of these early microvilli. At the plane of section illustrated, they could well be mere interdigitated pleats between adjacent receptive segments. More oblique profiles (not illustrated) establish that they are irregular tubules. Figures 9 and 10 establish interdigitation, from the relations between plasmalemmae, and the disposition of open microvillar bases in continuity with the cytoplasm of receptive segments.

3.2. *The ultrastructure of receptive segments from days* 10′–12*: the differentiation of microvilli, and conformational adjustments to the receptive segment mosaics*

By day 10′, irregular microvilli are present on the plasmalemmae of receptive segments throughout a retina. Figures 11 and 12, plate 7, show transverse sections of Layer I receptive segments. In figure 11, four segments each bear a single nascent rhabdomere. Figure 12 displays an entire foveal field at low magnification.

The pattern of tiering has also changed. Figure 13, plate 8, shows a transverse section through the far-distal field of foveal receptive segments, just proximal to the ‘pit’, and at the same transverse plane as the field shown for day 7′ by figure 3. Reading along a horizontal line from the outer side of the retina, 4a receptive segments are still juxtaposed to those of 3a. 3a receptive segments, however, are now adjacent to those of 4b, which lie on the inner side of a retina, indicating a substantial degree of differential axial migration.

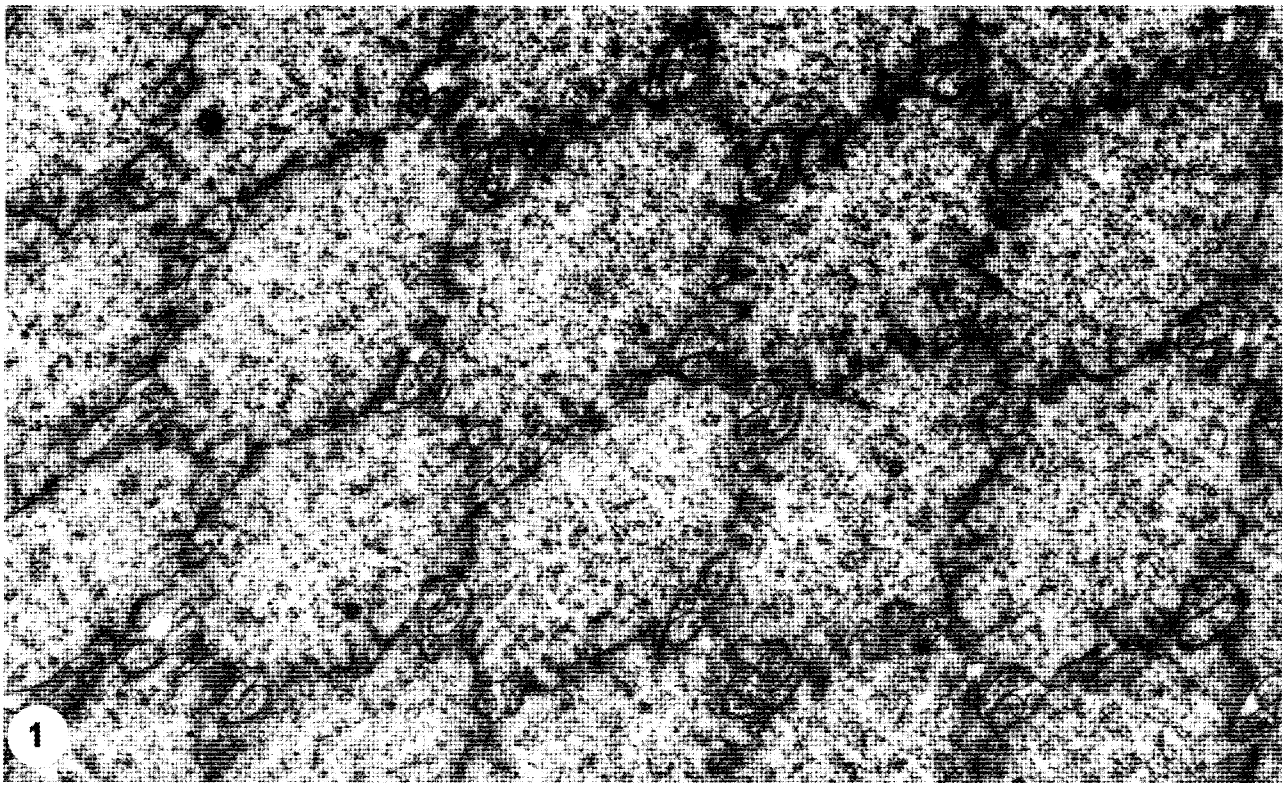
Figure 14, plate 8, illustrates peripheral Layer I receptive segments in the ventral arm of the retina. Plasmalemmae are almost entirely occupied by microvilli. In transverse section there are two discontinuities between the microvillar fields of a segment, anticipating the two rhabdomeres that a mature receptive segment will possess (see, for example, Blest & Price 1984).

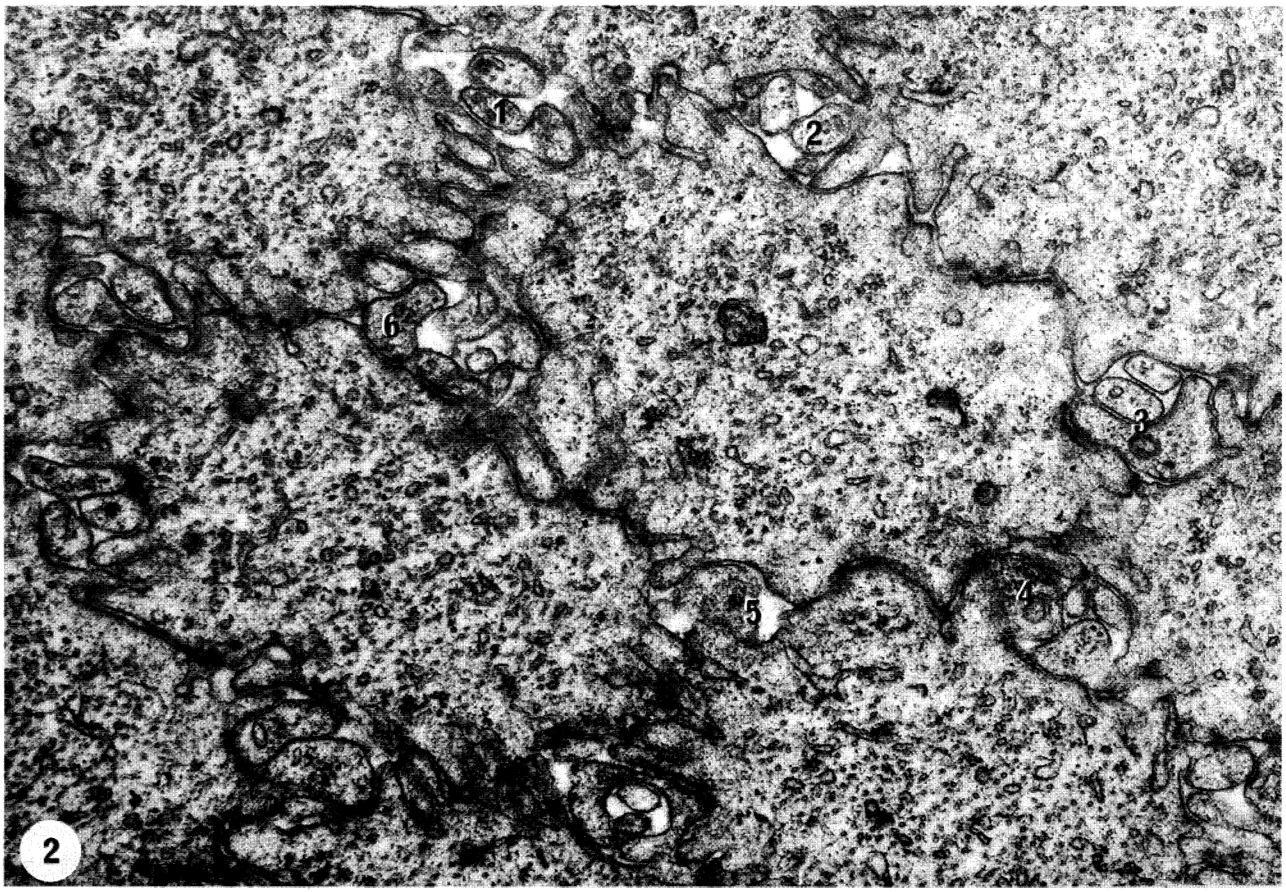
DESCRIPTION OF PLATE 1

Transverse sections of primordial receptive segments at day 3.

FIGURE 1. Receptive segments at the notional optical axis. (Magn. × 15000.)

FIGURE 2. Receptive segments in the peripheral retina. (Magn. × 30000.) The six glial strands that flank a receptive segment are labelled 1–6.





FIGURES 1 AND 2. For description see opposite.

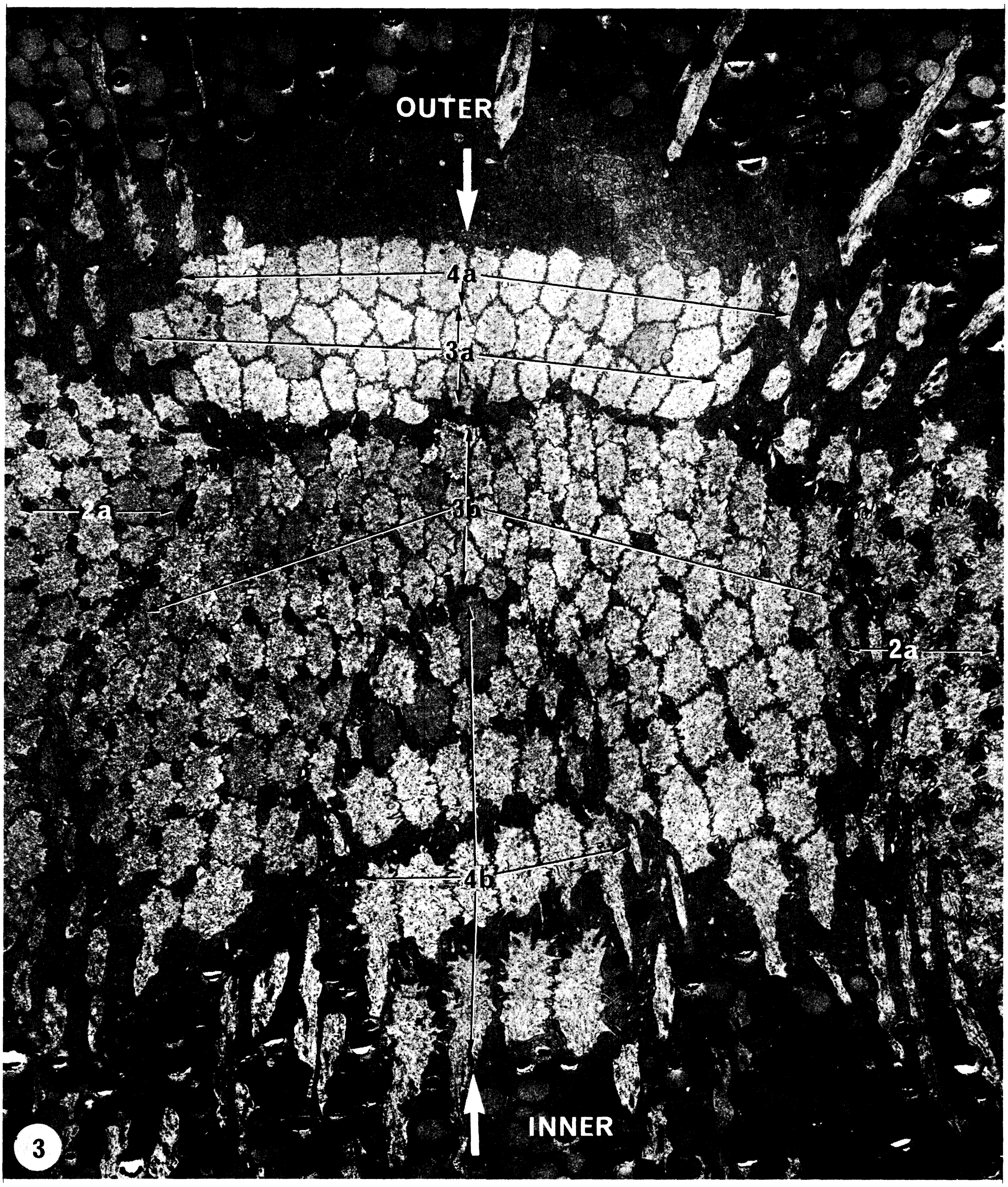


FIGURE 3. A transverse section of a principal retina at day 7′, taken just posterior to the apex of the developing 'pit'. Future receptive segments can be identified by their mosaic configurations and relative positions; their fields are labelled. Around the future optical axis, the outer side of the retina exhibits primordial Layer IV (4a) receptive segments. Adjacent to them, towards the inner side, are primordial 3a segments. The mid-region of the field is occupied by primordial 3b segments, and flanking them, on the inner side, primordial 4b segments. Ultimately, by day 10′, a single dorsoventral strip of 4a segments will be juxtaposed to the field of 4b segments (see figure 13). Peripherally, at day 7′, the foveal field illustrated is flanked dorsally and ventrally by primordial Layer II (2c) receptive segments. (Magn. × 3750.)

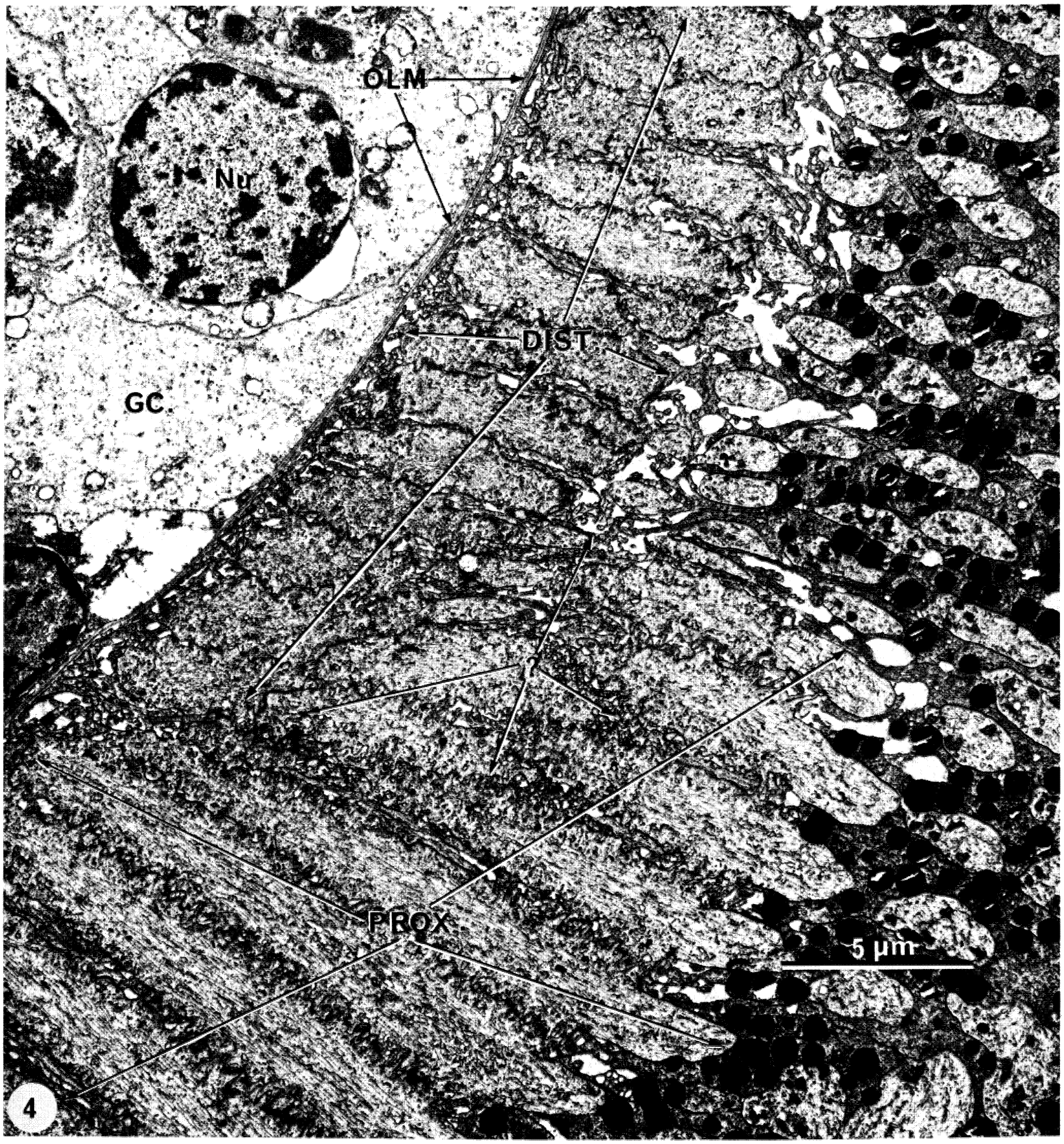


FIGURE 4. A sagittal section of a retina at day 7′. Primordial receptive segments are distinguished as proximal or distal with respect to future optical axis. It is evident that some degree of tiering has already been established. A region of primordial receptive segments is delineated as '?' because it is impossible to attribute them to a specific future Layer. (Magn. × 6250.)

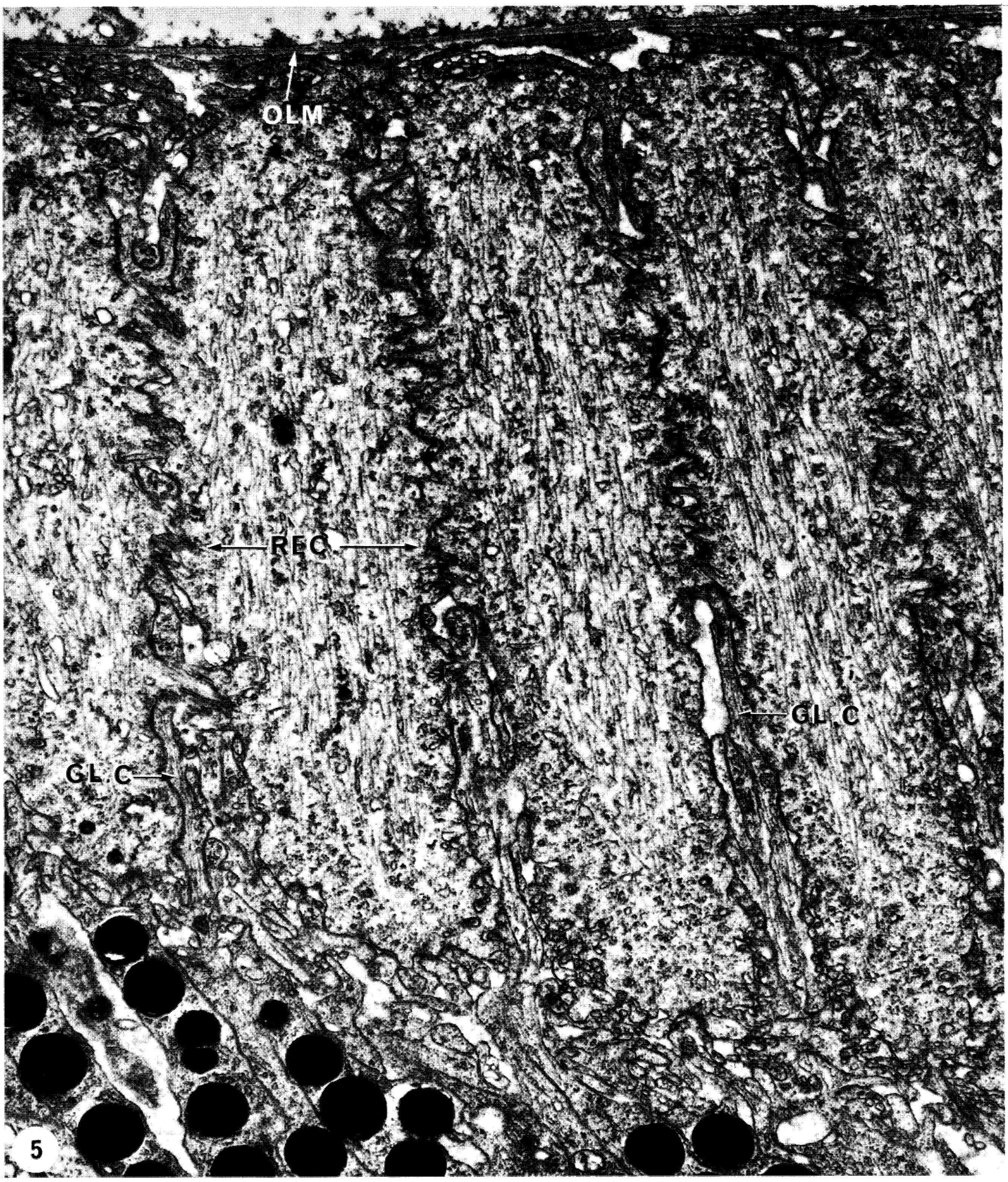
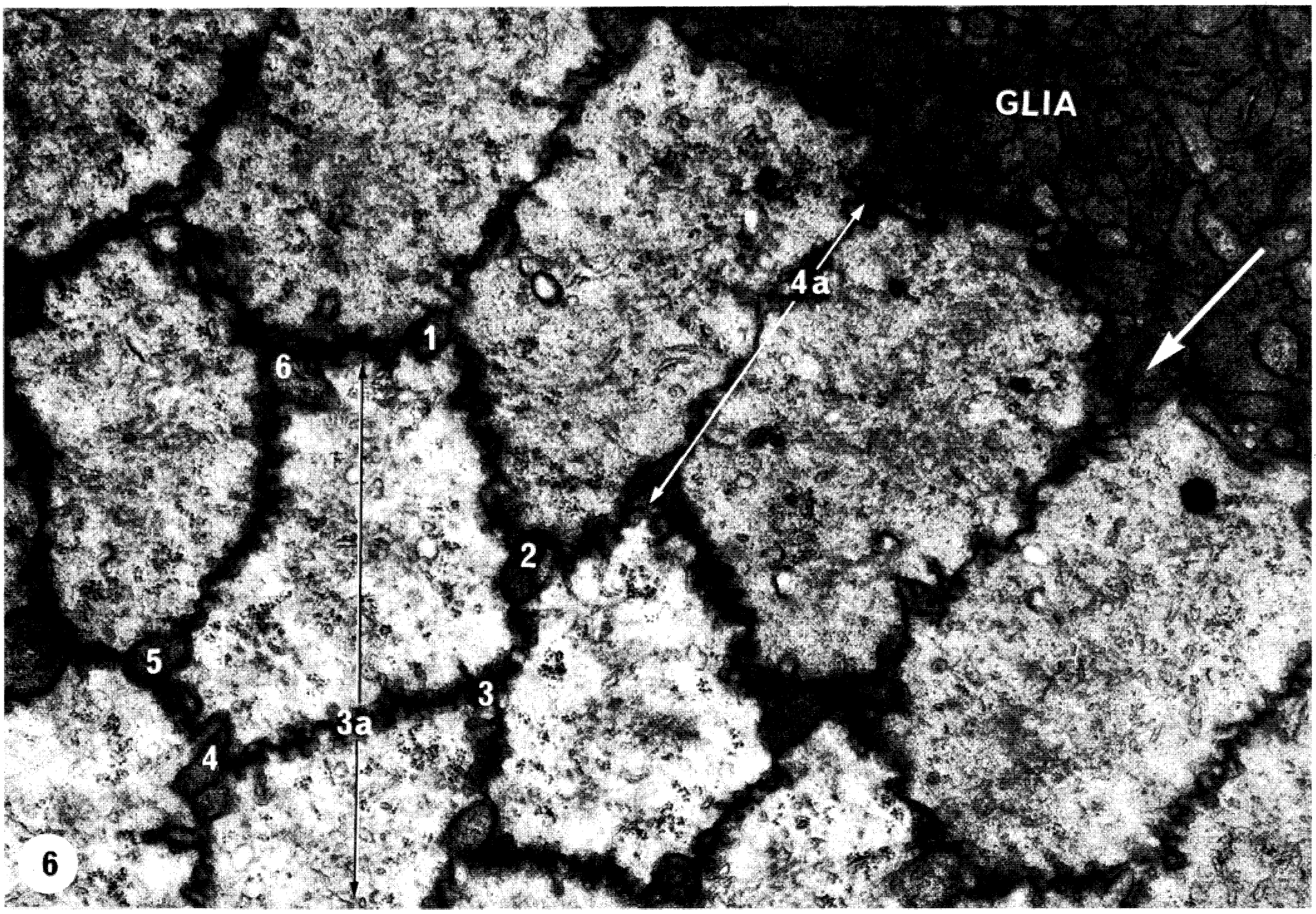
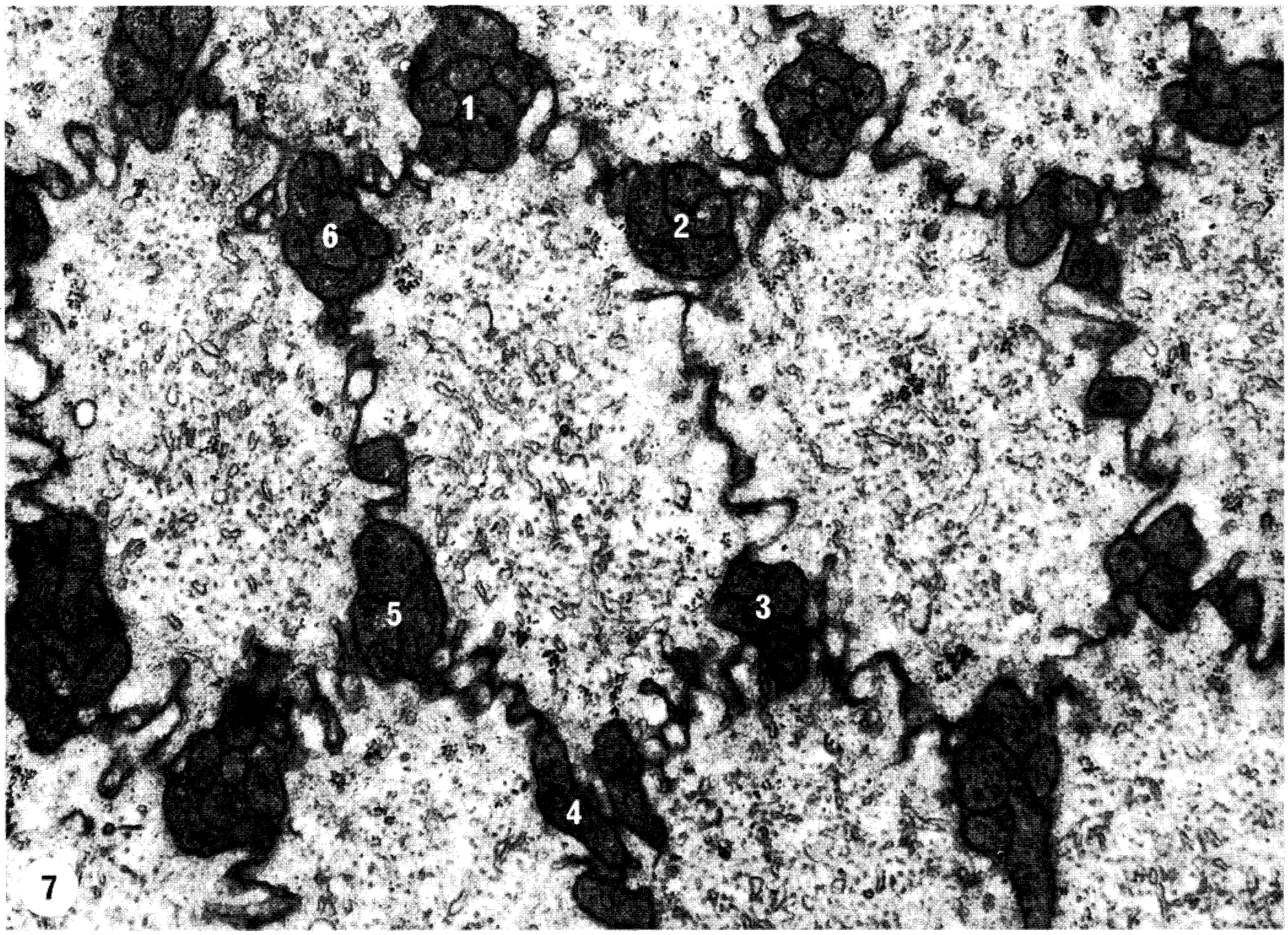
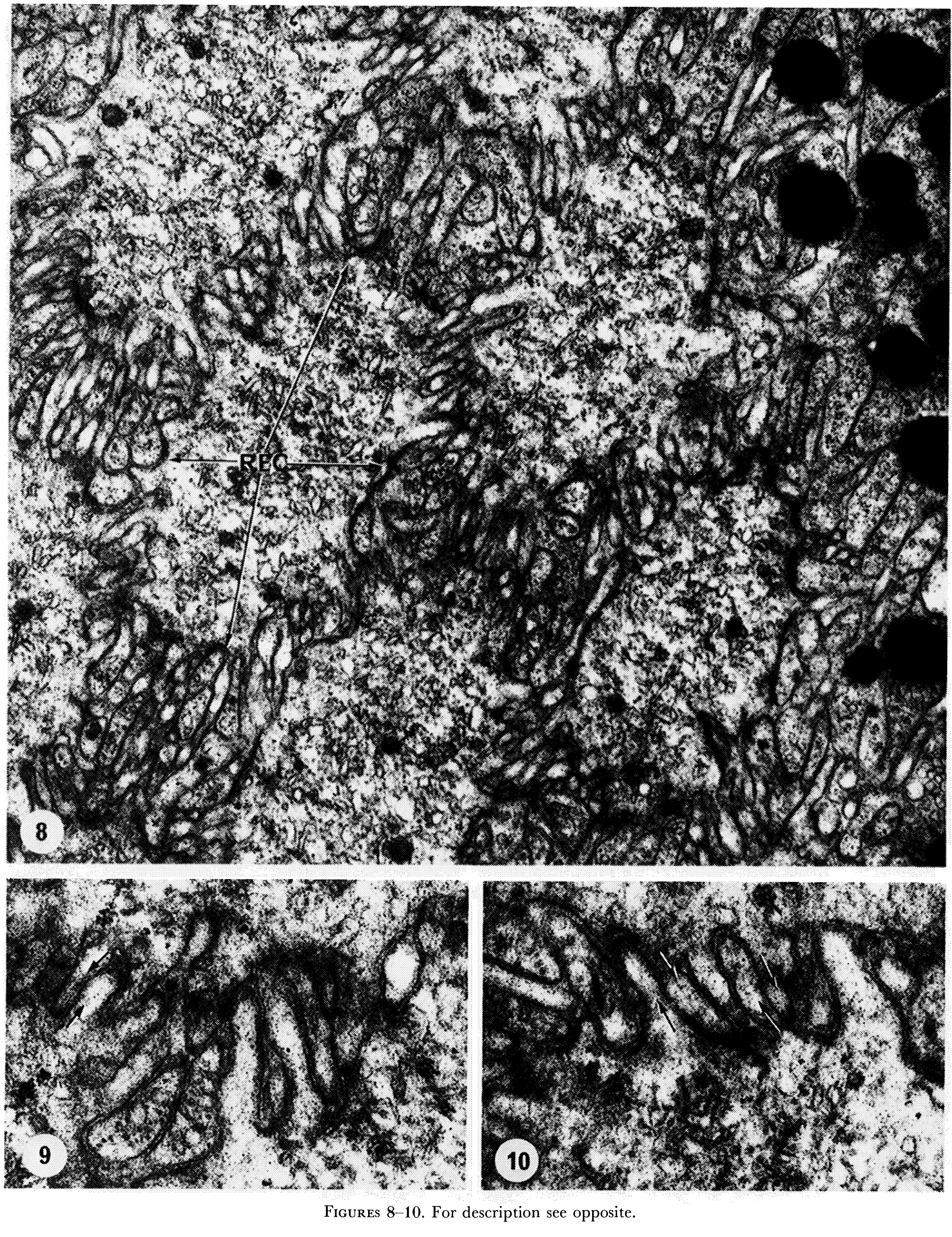


FIGURE 5. A sagittal section of primordial receptive segments near to the future optical axis, at day 7′.
(M 15000.)





Figures 6 and 7. For description see facing plate 6.



FIGURES 8–10. For description see opposite.

DESCRIPTION OF PLATE 5

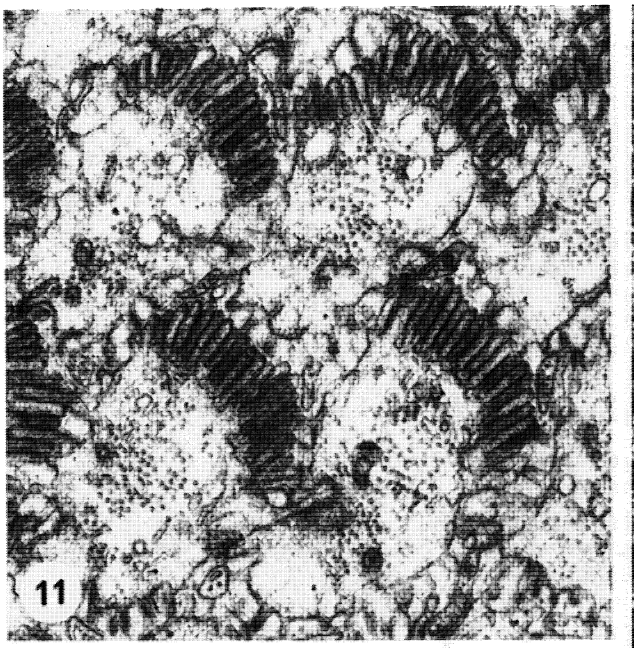
FIGURE 6. Transverse section of Layer III (3 a) and Layer IV (4 a) receptive segments near to the future optical axis at day 7′. (Magn. × 24000.)

FIGURE 7. Transverse section of Layer I receptive segments near to the future optical axis at day 7′. (Magn. × 24000.) In both figures glial strands are numbered 1–6 with respect to two individual receptive segments.

DESCRIPTION OF PLATE 6

FIGURE 8. Peripheral receptive segments in the ventral arm of a retina at day 7′, in transverse section. Irregular microvilli are distributed around their borders. (Magn. × 25000.)

FIGURES 9 AND 10. Ventral microvilli at day 7′, to indicate that early, irregular microvilli of contiguous receptive segments are interdigitated. Small arrows are directed at open microvillar bases. (Magn. × 50000.)



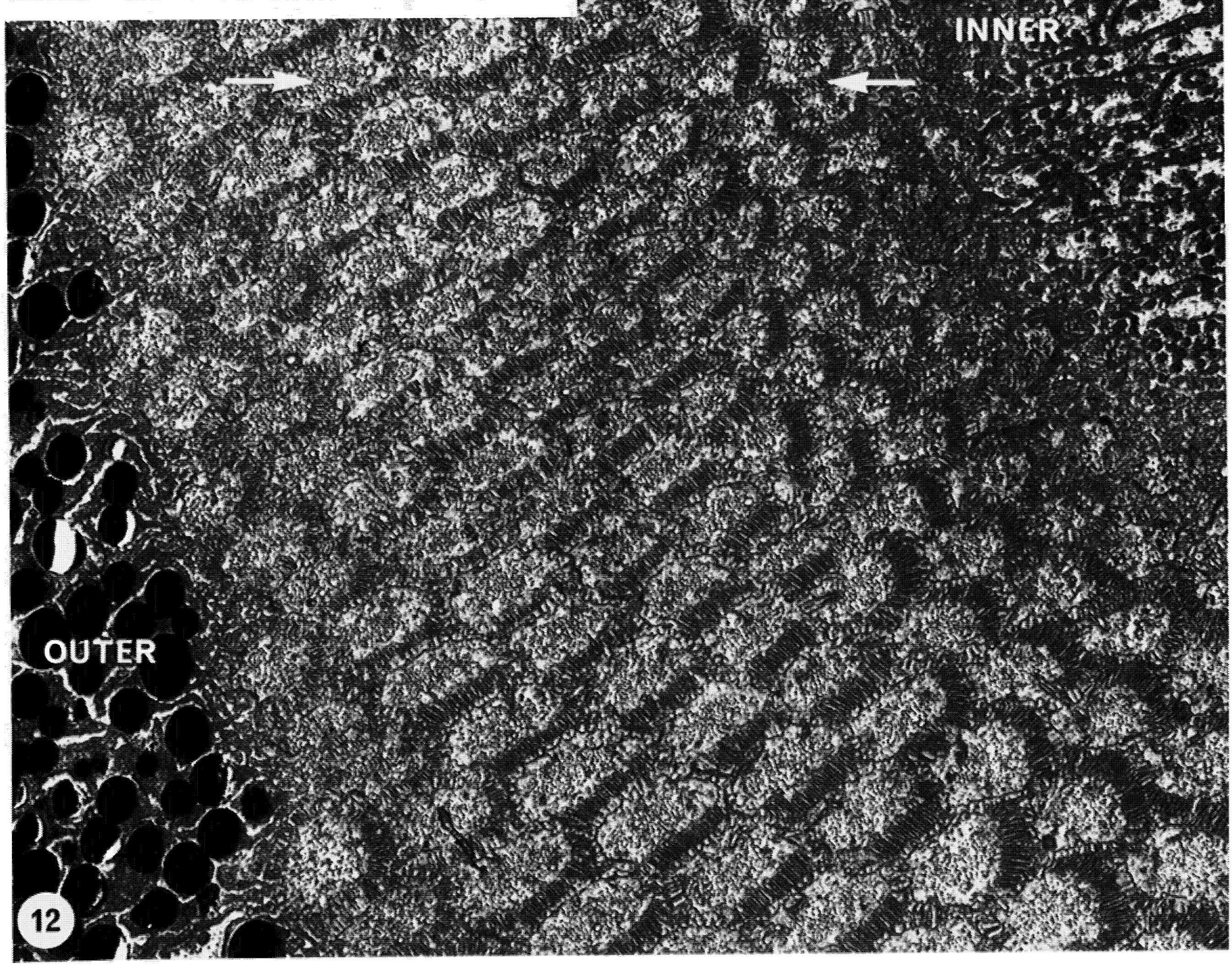
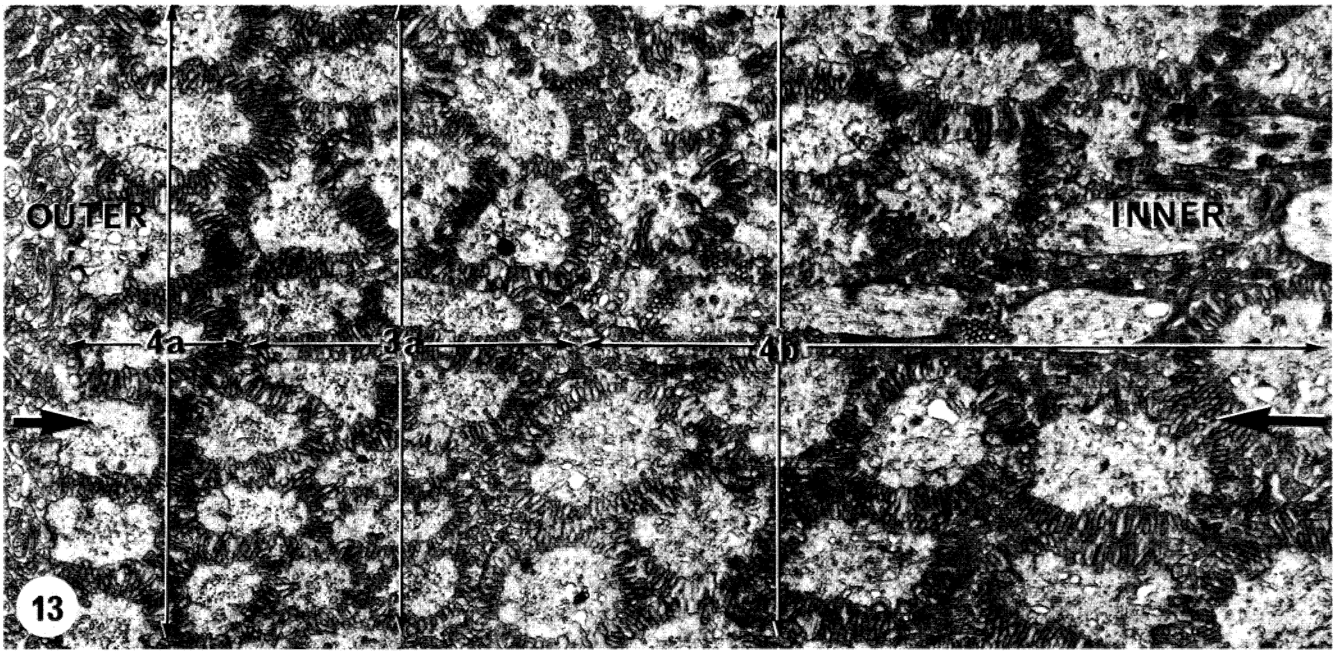


FIGURE 11. Transverse section of four Layer I receptive segments near to the future optical axis at day 10′. (Magn. × 25000.)

FIGURE 12. Low-magnification survey of the Layer I mosaic around the future fovea. (Magn. × 7500.)



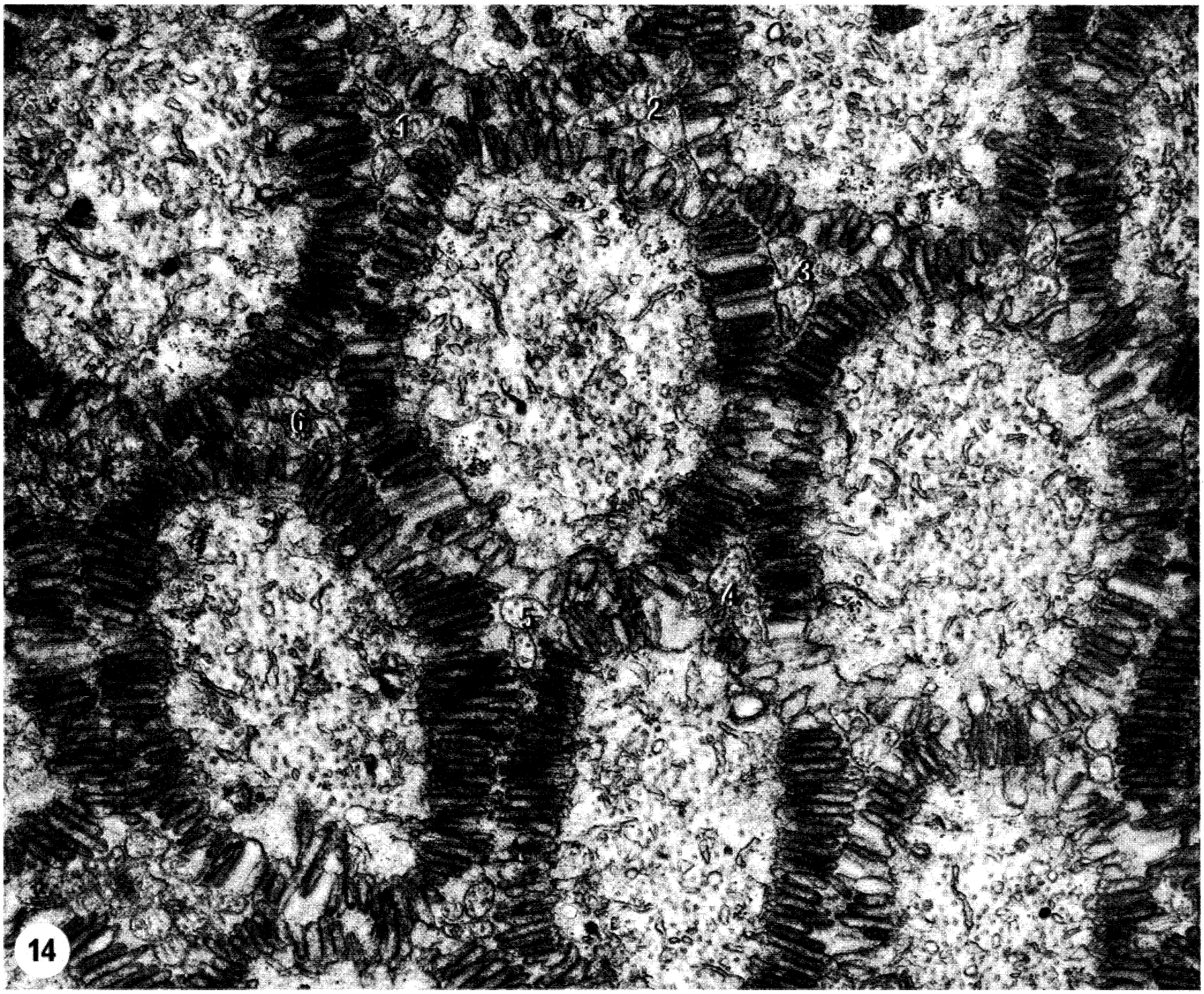


FIGURE 13. Transverse section of Layer IV (4a and 4b) and Layer III (3a) receptive segments, near to the fovea at day 10′. The plane of section is identical to that in figure 3. (Magn. × 10500.)

FIGURE 14. Transverse section through either Layer I or Layer II (2c) receptive segments in the peripheral ventral arm of a retina, at day 10′. (Magn. × 25000.)

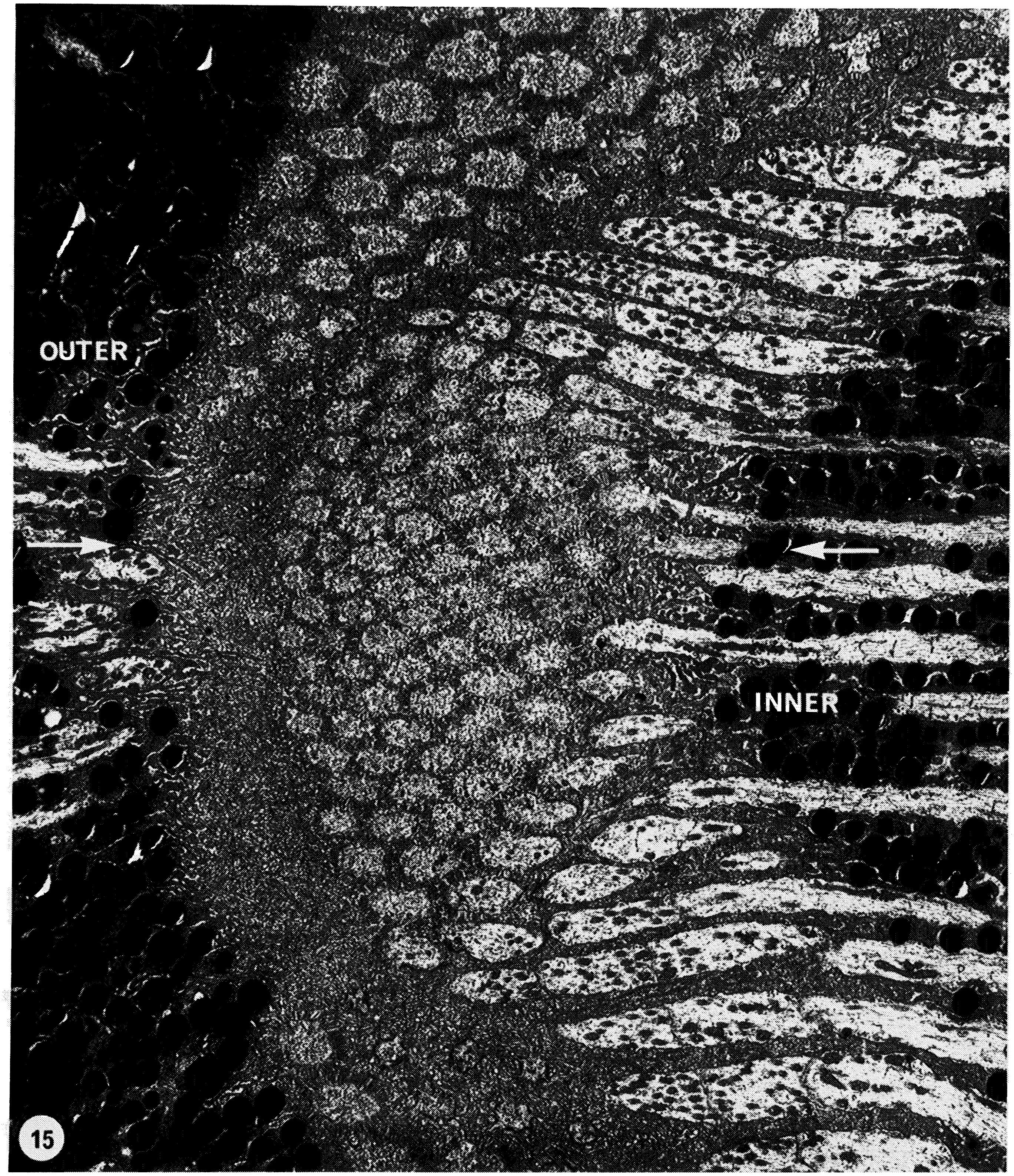


FIGURE 15. Low-magnification survey of the Layer II (2b) receptive segments mosaic, in transverse section, at day 10′. (Magn. × 5000.)

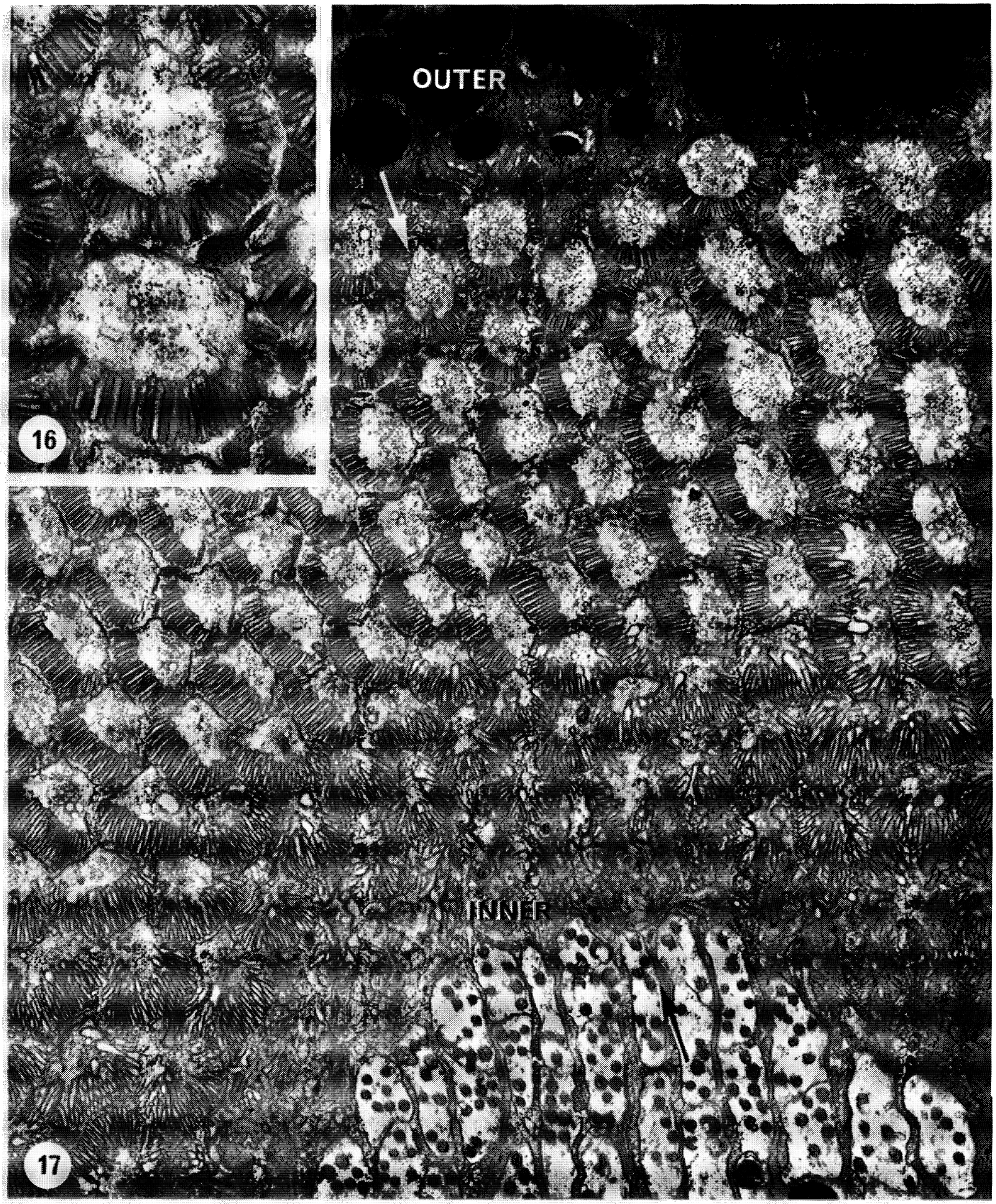


FIGURE 16. Transverse section of two Layer I receptive segments near to the fovea at day 12. (Magn. × 25000.)

FIGURE 17. Low-magnification survey micrograph of foveal Layer I receptive segments in transverse section, at day 12. (Magn. × 10000.)

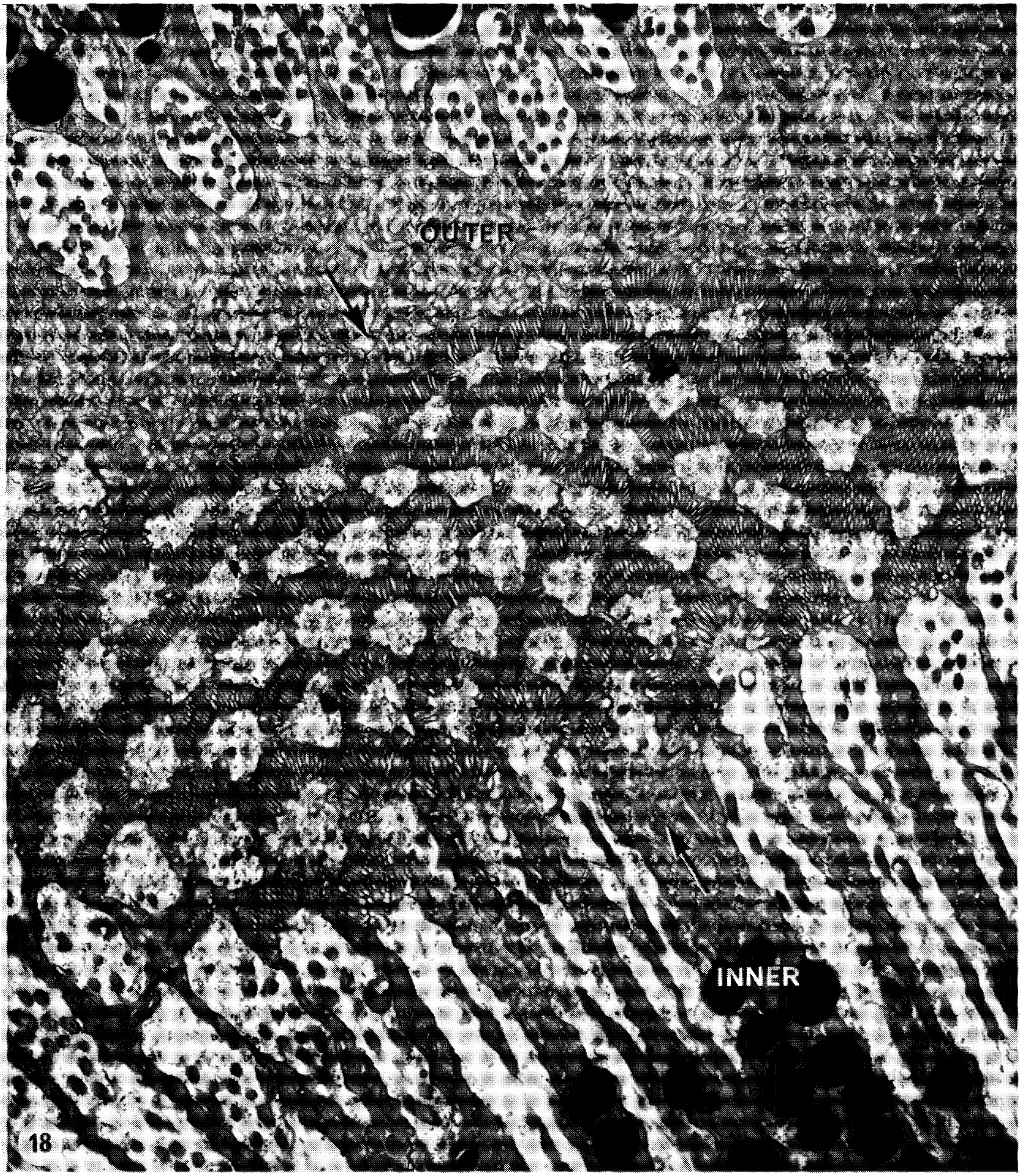


FIGURE 18. Low-magnification survey micrograph of Layer II (2b) receptive segments around the fovea, in transverse section, at day 12. (Magn. × 10000.)

FIGURE 19. Low-magnification micrograph of Layer IV (4a and 4b) receptive segments around the fovea, in transverse section, at day 12. (Magn. × 12500.)

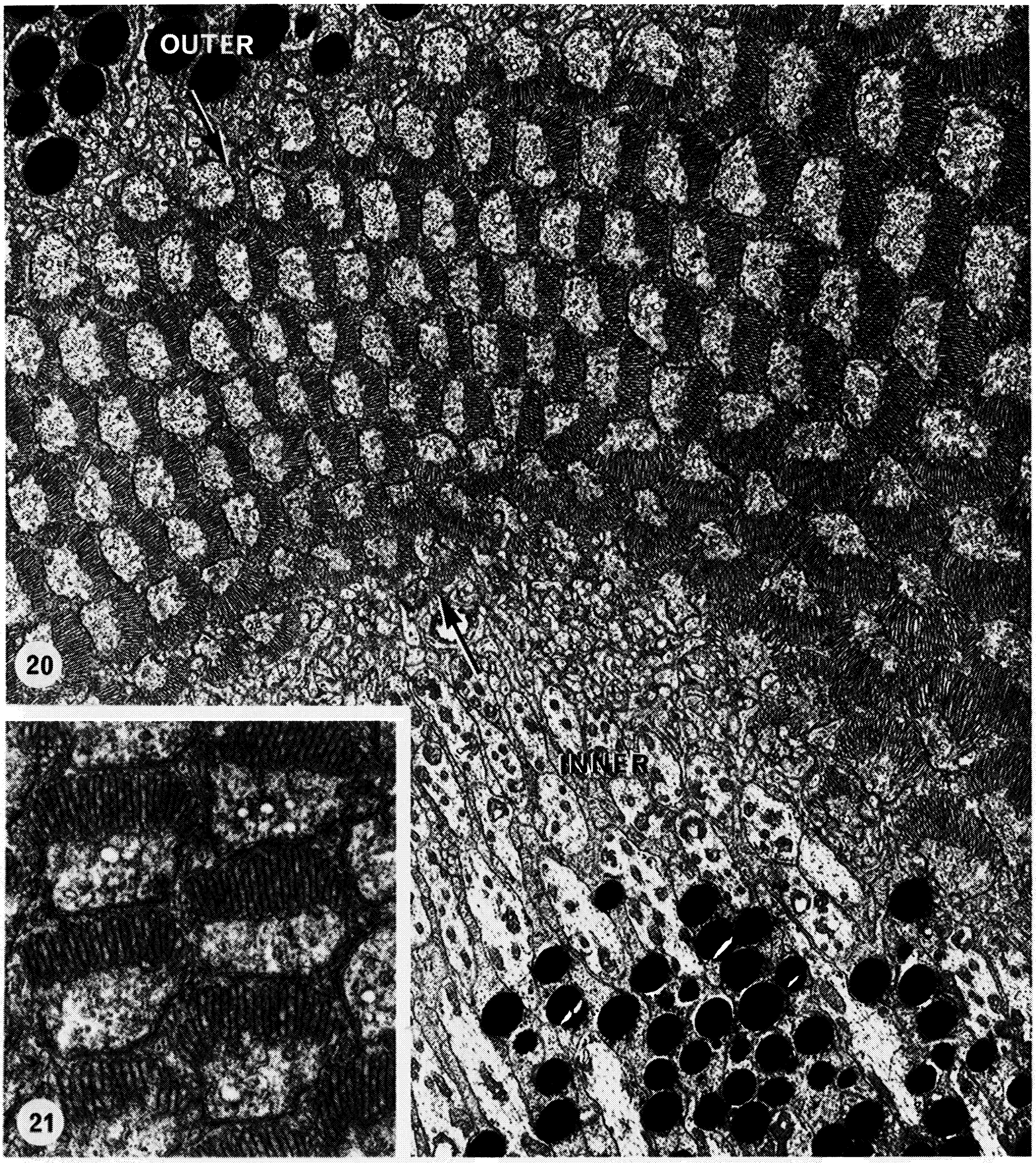


FIGURE 20. Transverse section of Layer I receptive segments around the fovea, at day 13. (Magn. × 8750.)

FIGURE 21. Transverse section of Layer I receptive segments near to the fovea at higher magnification. (Magn. × 25000.)

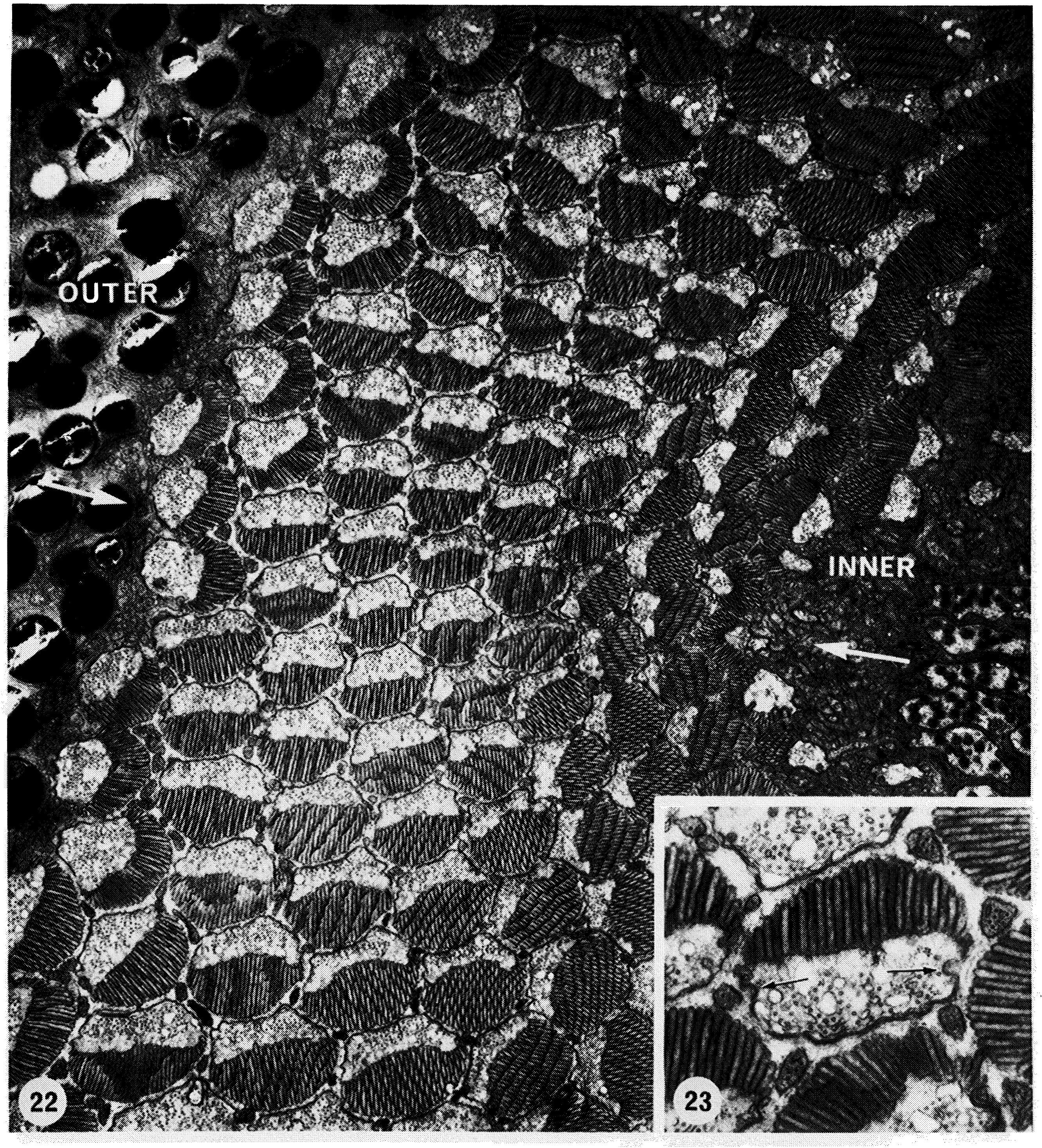


Figure 22. Low-magnification survey micrograph of Layer I receptive segments near to the fovea, in transverse section, at day 14. (Magn. × 10500.)

Figure 23. Transverse section of a single Layer I receptive segment at day 14. Two endocytotic pits are indicated by small arrows. (Magn. × 25000.)

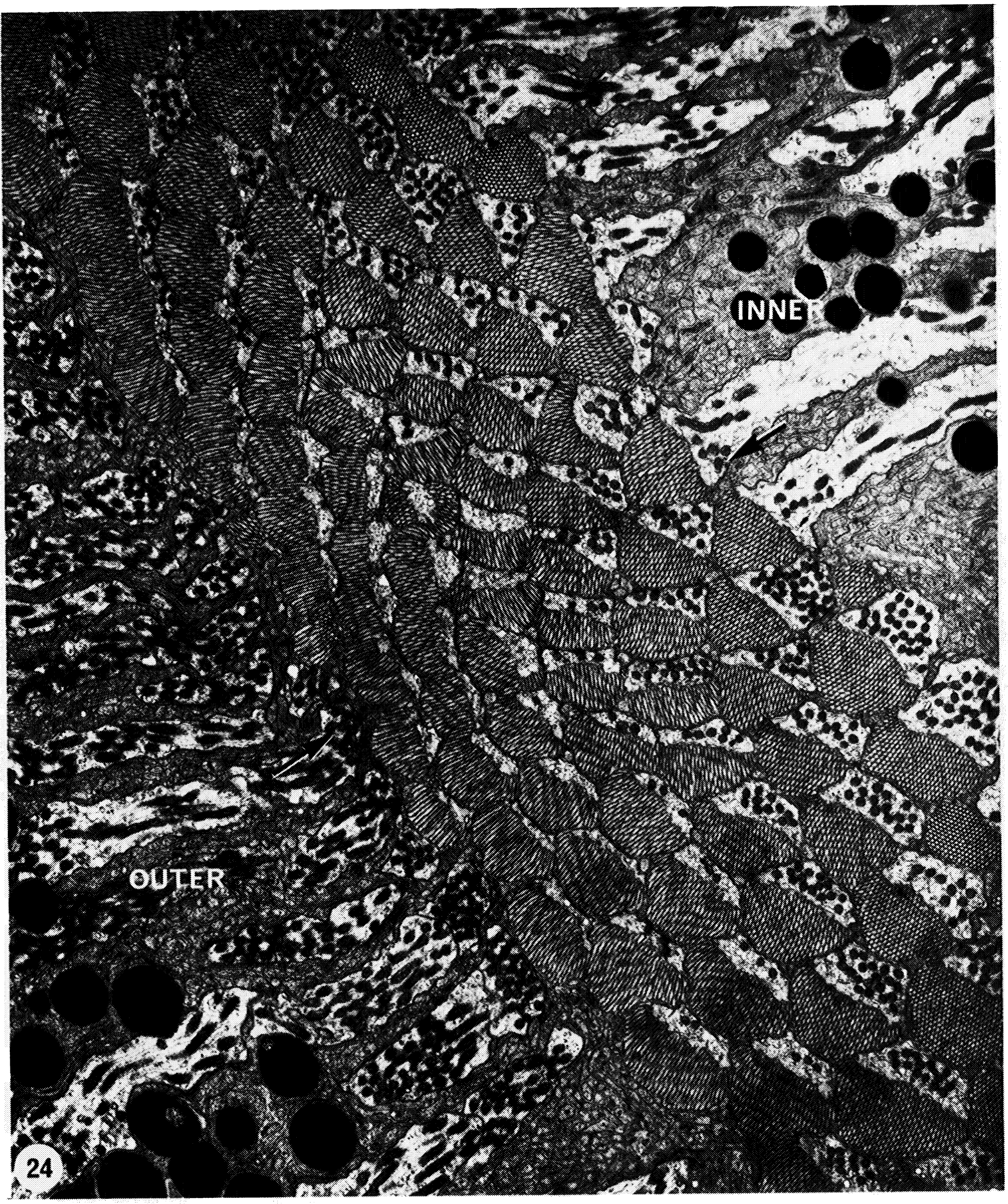


FIGURE 24. Low-magnification survey of Layer II (2b) receptive segments around the fovea, in transverse section, at day 14. (Magn. × 10000.)

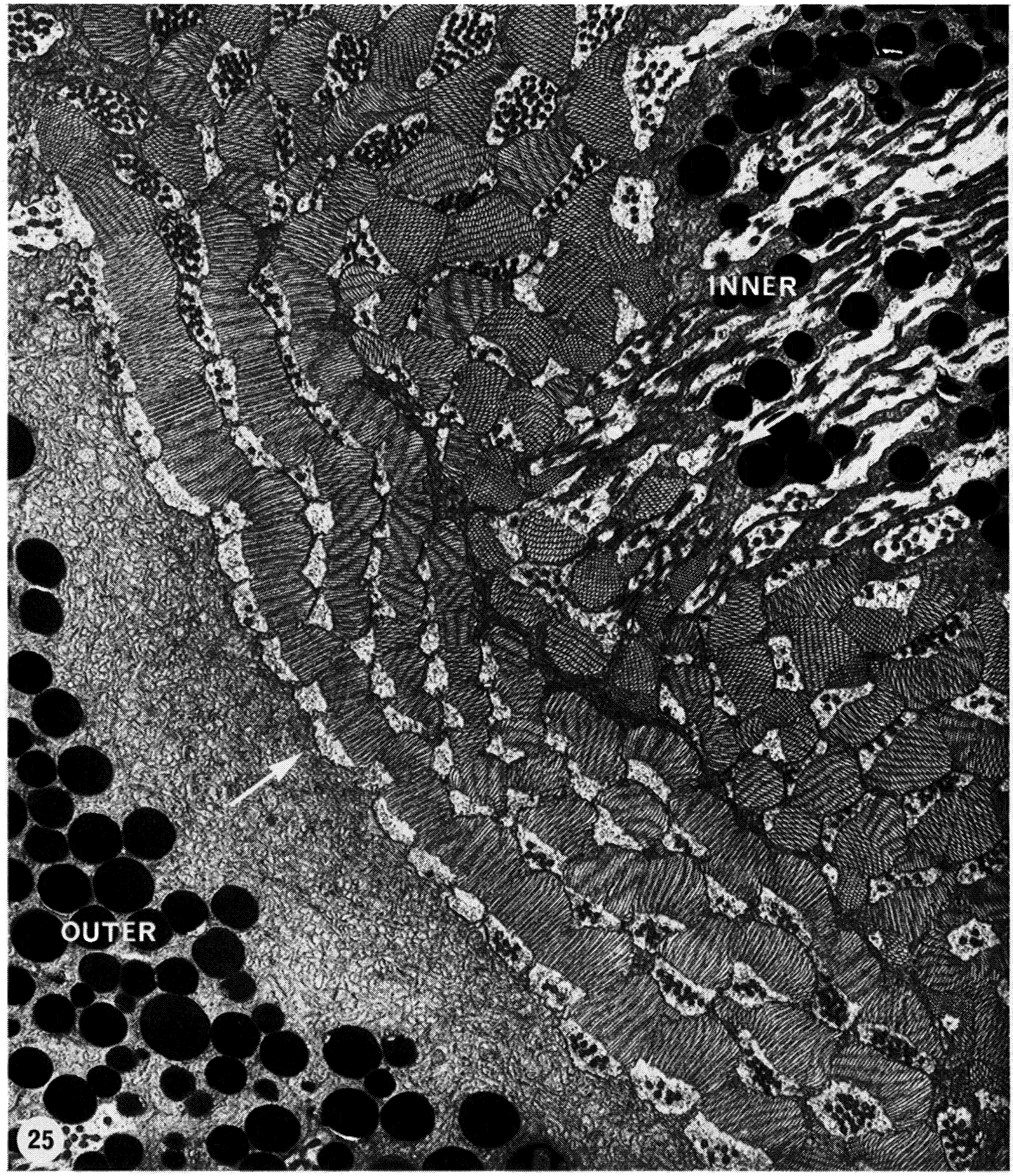


FIGURE 25. Low-magnification survey of Layer III (3a and 3b) receptive segments around the fovea, in transverse section, at day 14. (Magn. × 6150.)

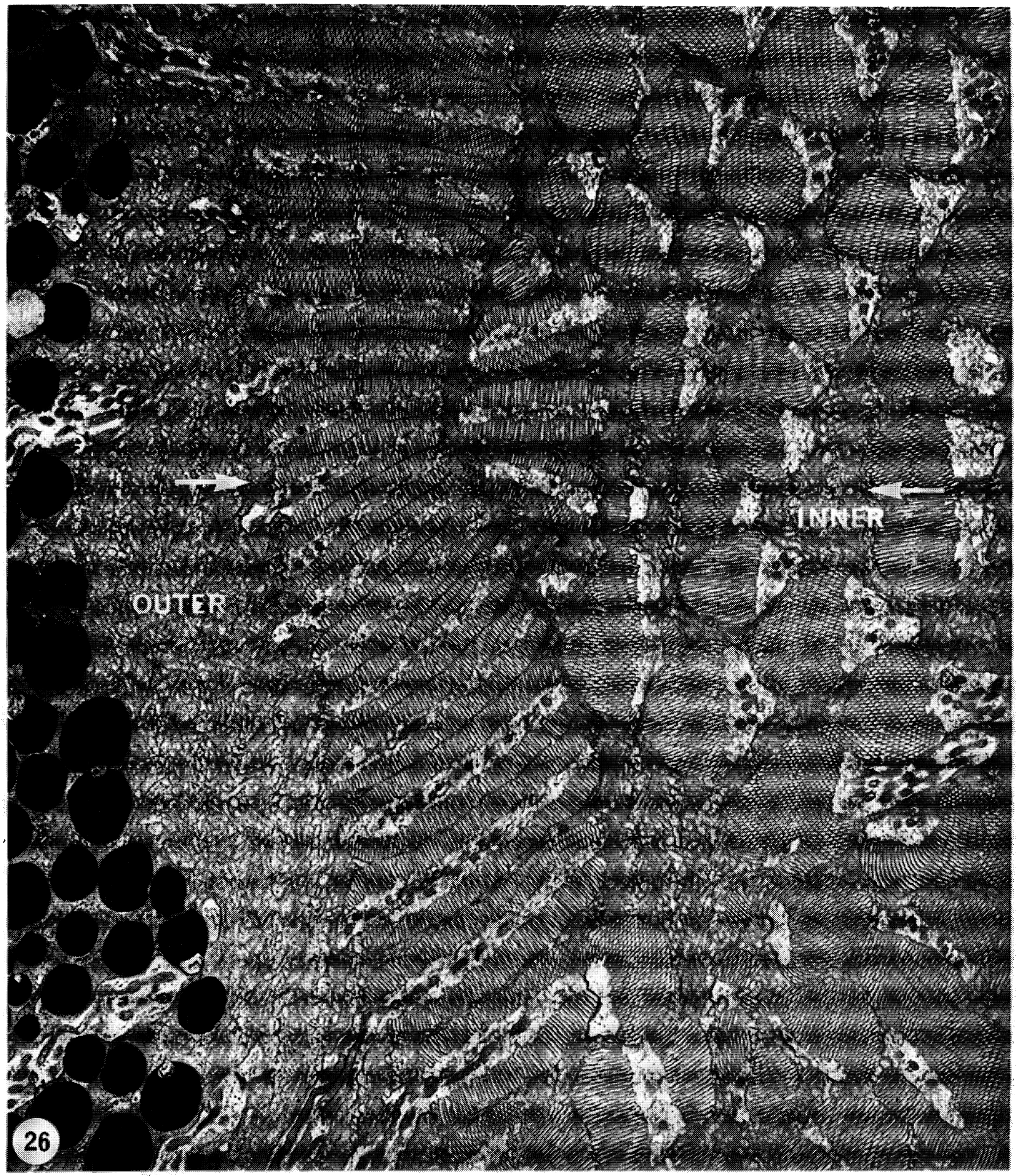


FIGURE 26. Low-magnification survey of Layer IV (4a and 4b) receptive segments around the fovea, in transverse section, at day 14. (Magn. × 8750.)

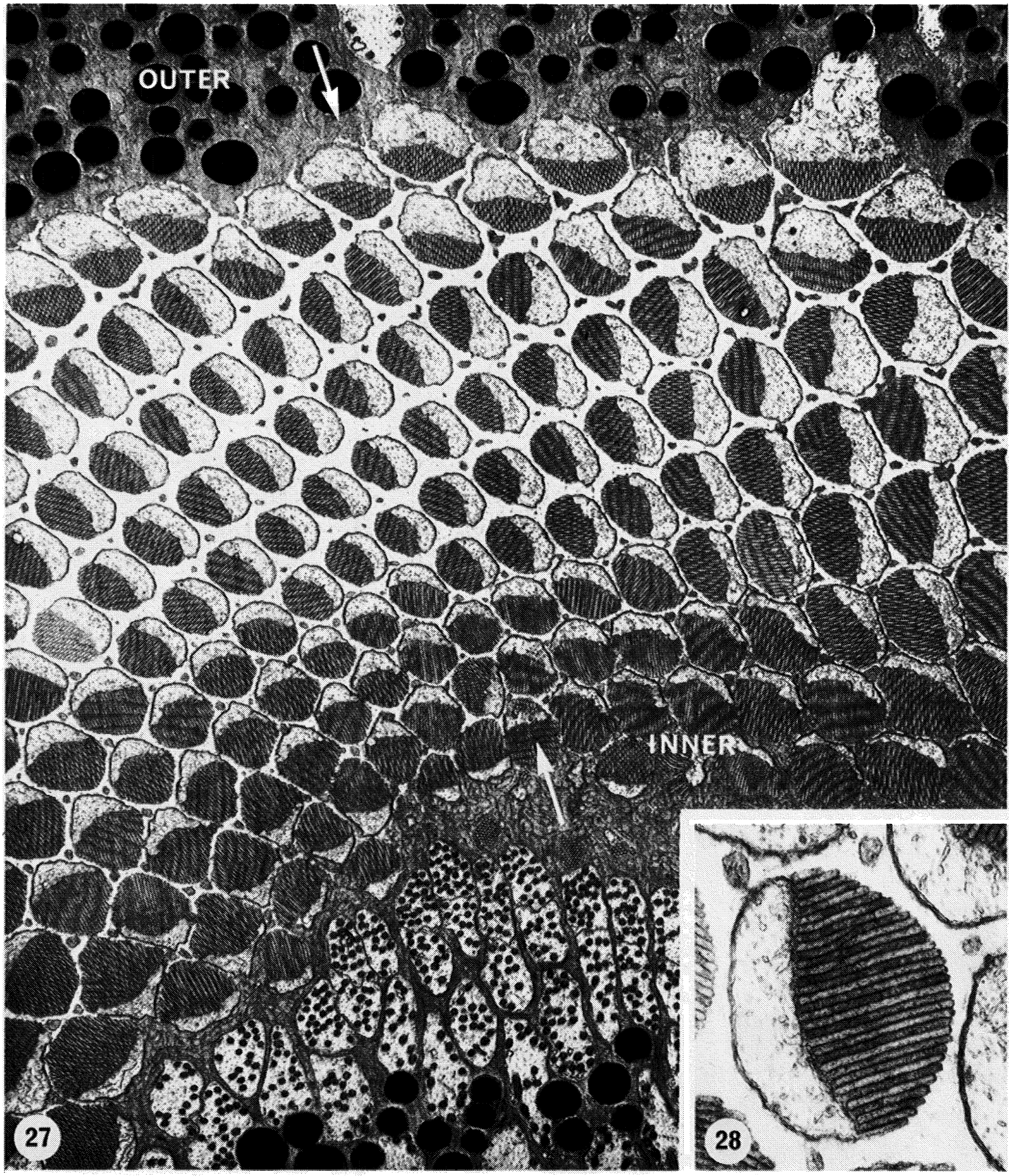


FIGURE 27. Low-magnification survey of Layer I receptive segments around the fovea, in transverse section, at day 15. (Magn. × 7500.)

FIGURE 28. Single Layer I receptive segment near to the fovea, in transverse section, at day 15. (Magn. × 25000.)

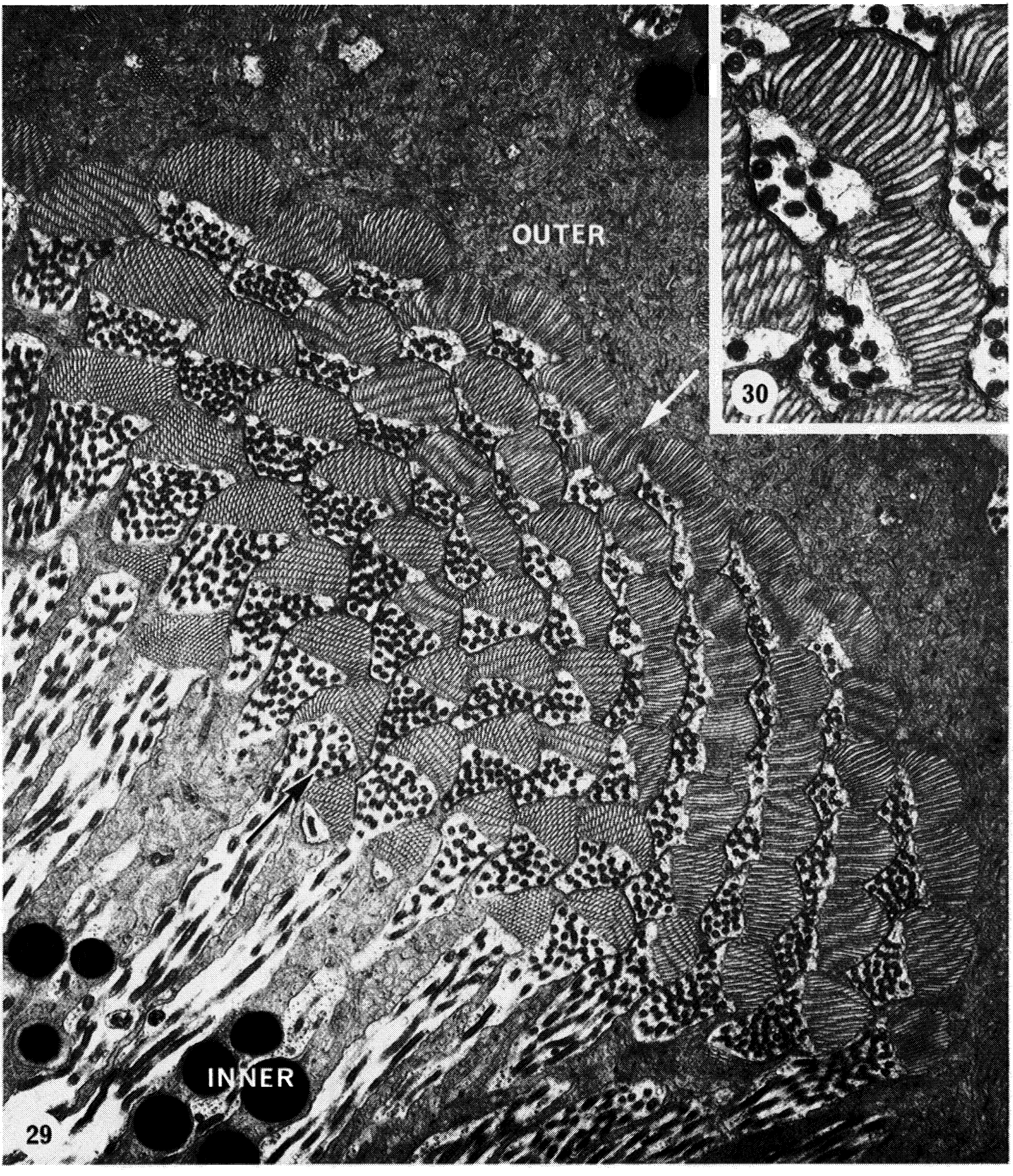


FIGURE 29. Low-magnification survey of Layer II (2b) receptive segments around the fovea, in transverse section, at day 15. (Magn. × 8750.)

FIGURE 30. Layer II (2b) receptive segments with confluent rhabdomeres, in transverse section, at day 15. (Magn. × 25000.)

Figure 15, plate 9, gives an entire transverse profile of Layer II (2b) receptive segments at low magnification, to show that the characteristic fan-shaped arrangement is already established at day 10′.

Surprisingly, at day 12, just after the moult to the second instar, receptive segments have still not reached their final states. Figure 16, plate 10, shows two foveal Layer I receptive segments. Microvilli are concentrated on one face of the plasmalemma, but they are still short, relatively irregular and wide. They may be compared with the mature arrangement shown by figure 28, plate 18, for an equivalent receptive segment at day 15. Figure 17, plate 10, displays an entire foveal Layer I array at day 12. Figure 18, plate 11, offers a transverse section through a Layer II (2b) mosaic, and figure 19, plate 12, through the Layer IV (4a and 4b) mosaics. It is evident that receptive segments have not yet acquired their final architectures from the following.

1. Microvilli are not regular, nor do they occupy the volumes of their receptive segments that they will ultimately achieve. Thus, for example, the cytoplasm of Layer IV (4a and 4b) receptive segments is relatively voluminous, and is densely occupied by organelles.
2. The condensed mitochondria that occupy the cytoplasm of Layer II–IV receptive segments have not yet acquired their final population densities.

3.3. *The ultrastructure of receptive segments after the moult to the second instar at day* 12

Days 13 and 14 exhibit progressive changes to the receptive segments of all four Layers (plates 13–17). Substantial growth and differentiation of the rhabdomeres of foveal Layer I take place, for example, between day 13 (figures 20 and 21, plate 13,) and day 14 (figures 22 and 23, plate 14). During day 14, the plasmalemmal areas of foveal Layer I receptive segments are reduced, so that the previous virtual contiguity between them is eliminated. Figure 23 illustrates the endocytosis of plasma membrane by which this is achieved: two endocytotic pits lie one on each side of the rhabdomere. The final differentiation of Layer I lags slightly behind that of Layers II–IV, which by day 14 have almost attained their mature states (figures 24–26, plates 15–17).

Figures 27 and 28, plate 18, illustrate the final state of foveal Layer I at day 15. The retina was sampled from a spiderling that had just spontaneously departed from a brood sac. There are substantial lacunae between receptive segments, spaces being occupied by a diffuse, structureless matrix, not visible in the two micrographs chosen for plate 18. The glial strands are radically reduced in thickness, and in some regions have been eliminated altogether. The rhabdomeres are now large, approximately oval in cross section, and composed of slender microvilli of uniform diameter (*ca.* 57 nm).

Figures 29 and 30, plate 19, allow the state of Layer II (2b) at day 15 to be compared with the same region at day 14 (figure 24). The arrangement of microvilli and mitochondria is identical to the adult dispositions described for an advanced salticid by Blest & Price (1984).

3.4. *A note on the reorganization of the glial matrix and of the glass cells*

The description of progressive changes to the ultrastructure of the developing receptive segments (§§3.1–3.3) implies that the final mosaics are generated at least in part by the local growth or involution of processes of the pigmented glia.

The shape of the interface between the glial matrix and the contents of the retinal tube is also functionally critical (see, for example, Williams & McIntyre 1980; Blest *et al.* 1988). It is not at present clear how the shape of the interface is determined, but it is not realized until after the moult to the second instar at day 12 (Blest 1988*b*). It could result purely from morphogenetic events private to the glial processes, or from an interaction between the forming glial matrix and the glass cells.

4. INTERPRETATION

4.1. *The differentiation of rhabdomeral microvilli*

The major conformational transformations that establish retinal tiering over the first seven days of post-embryonic development (Blest 1988*b*) precede the differentiation of rhabdomeral microvilli.

Microvilli first appear as irregular, interdigitated pleats between contiguous receptive segments. Interdigitation of microvilli between adjacent segments is a feature of the secondary eyes of many spiders (Blest 1985*a*). Later, all receptive segments display microvilli of more conventional shape that are not interdigitated. Nevertheless, they are irregular and, in the micrographs that illustrate this account, rather poorly preserved for electron microscopy. Satisfactory histological preservation of microvilli only occurs at around day 15 (figure 28).

We have provided pharmacological evidence that poor fixation of rhabdomeral microvilli for electron microscopy can result from the activation of Ca^{2+}-dependent cysteine proteases during primary fixation (Blest *et al.* 1982; Blest *et al.* 1984; Blest & Sigmund 1985*b*). In mature Salticidae, excellent fixation of rhabdomeral microvilli can be obtained without the use of such special strategies as chelating Ca^{2+} ions from primary fixation media, providing a supplement of Mg^{2+} ions at the same stage of processing, or inhibiting cysteine proteases before primary fixation (see, for example, Blest & Maples 1979; Blest & Price 1974; Blest & Sigmund 1985*a*, and figure 28 in this paper). It remains to be discovered whether or not poor fixation of microvilli in post-embryonic retinae until day 15 results from a complement of proteases whose impact diminishes as the receptive segments differentiate, or whether there is some other explanation.

The rapid increase in the topological order of the microvillar arrays between days 13 and 15 is so well displayed by micrographs (figures 21, 23 and 28) that it is unnecessary to qualify it with measurements. It implies that there must be radical changes to the molecular architecture of the microvilli as they mature. How the regular (and optically critical) arrays of rhabdomeral microvilli are generated and maintained in arthropods is still an open question (reviewed by Blest (1988*a*)). A major problem relates to the ambiguities that still surround the nature of the microvillar cytoskeleton, because it can be supposed, *a priori*, to contribute to the stability of a microvillar array, and, possibly, to determine how it is erected in the first place. Here, we can only note that the final transformations between days 13 and 15 are dramatic, and suggest many molecular problems that we are in no position to resolve.

4.2. *Optical goals, and how they are realized*

Our modelling of the optical performance of principal eyes (Williams & McIntyre 1980; Blest *et al.* 1981; Blest *et al.* 1988) indicates that several parameters must be precisely determined during development if an eye is to work effectively. Some are accessible by the present study, others are not. Of the latter, (*a*) precise regulation of the shape of the corneal lens is critical; and (*b*) the axial dimensions of the dioptrics are crucial to the performance of the retina, because they specify the transverse planes at which focused images will be received by the mosaics of receptive segments. Similarly, (*c*) the diverging component of the telephoto system provided by the 'pit' must be an exact hemisphere, and our present data do not tell us how it is attained.

We can, however, look at the development of the foveal Layer I mosaic in terms of what it is 'designed' to do, and scrutinize the more distal Layers in the same way.

Images must be focused on the distal tips of the 'staircased' rhabdomeres of Layer I, and transmitted down them as pixels by internal reflection. The ultrastructure of Layer I receptive segments cannot allow this mechanism to operate until day 15. Beforehand, foveal Layer I receptive segments are contiguous, and their cytoplasm is so densely occupied by organelles as to make it unlikely that the refractive index difference between a rhabdomere and its cytoplasmic surround could permit it to perform as a light guide. Between days 13 and 14, and day 15, organelles are deleted from the cytoplasm of foveal Layer I receptive segments, leaving a population of microtubules, and rhabdomeres grow to their mature dimensions. The plasmalemmal area of a receptive segment is reduced by endocytosis, so that foveal Layer I receptive segments are, at day 15, well separated from each other. The degree of separation has previously been observed for the Layer I receptive segments of second instar spiderlings of *Phiale magnifica* (Blest 1987). It is not found in adult, advanced salticids (see, for example, Blest & Price (1984), and A. D. Blest, unpublished observations) including adult *Plexippus*. In such mature retinae, foveal Layer I receptive segments are closely contiguous, and light guiding can be supposed to be assured by the refractive index difference between a rhabdomere and its cytoplasmic surround alone (Williams & McIntyre 1980; Blest *et al.* 1981).

Thus at day 15, a Layer I receptive segment is an isolated cylinder. If the refractive index of its cytoplasm is sufficiently close to that of a rhabdomere, the entire segment could act as a light guide. More probably, light guiding is a function of a rhabdomere acting by itself.

Similarly, equilibration of the refractive indices of the rhabdomeres and cytoplasmic surrounds of Layers II–IV rhabdomeres, which we have suggested to be a device to minimize the degradation of images received by Layer I at the fovea (Blest *et al.* 1981), cannot be complete until around day 14, when the cytoplasm has acquired a full complement of mitochondria.

4.3. *Phylogenetic implications*

An accompanying discussion (Blest 1988*b*) assumes that an ontogenetic sequence can be a repository representing the phylogenetic events that led to it, but also emphasizes that such sequences cannot be interpreted too literally, for reasons that are now well established. The paradigm of ontogenetic recapitulation seems plausible as applied to the gross conformational changes undergone by a principal retina as it develops: how far can we go in the context of the ultrastructural development of individual receptive segments? Do the successive transformations that they undergo recapitulate anything, and what can we infer if they apparently do so?

1. The sequence of changes undergone by individual microvilli soon after they appear to a remarkable extent parallels the condition of microvilli in less sophisticated araneomorph spiders (Blest 1985*a*).

2. Initially, the microvilli of contiguous receptive segments are interdigitated. Interdigitation has been noted for the adjacent receptive segments of several 'primitive' araneomorph spiders (Blest 1985*a*; Blest & Sigmund 1985*b*).

3. Interdigitation is not found in various more ‘advanced’ araneomorph spiders: for example, pisaurids (Blest & Day 1978) and argyopids (Uehara *et al.* 1977, 1978).

4. Naïvely, it could be supposed that the growth and reorganization of microvilli during the early stages of post-embryonic development recapitulate a sequence of phylogenetic events. They may, but, if they do, there are some important caveats: why cannot such a laborious sequence of events be ‘telescoped’ so as to eliminate redundant steps? Why does it all take so long?

Obviously, as long as post-embryos remain within their brood sacs they have no reason to see anything. After they moult to the second instar, the new cuticle needs time to harden, but to delay finalization of the visual apparatus until spiderlings spontaneously become independent seems selectively extravagant. How well do they cope with the outside world if, for example, a brood sac is disturbed by a predator and they are forced to leave it prematurely?

5. The highly ordered microvilli of foveal Layer I receptive segments are unique among spiders (Blest 1985*a*). The narrow diameters of the microvilli (*ca.* 57 nm) and their uniformity are probably only found elsewhere in the photoreceptors of higher Diptera, which also sustain an extremely sophisticated visual performance (see Snyder (1979) for a review of the relevant optical theory).

Thus in a broad sense, the sequence of morphogenetic changes to the receptive segments might be supposed to present a parade of phylogenetic events. In detail, the concept will probably prove simplistic. Blest (1985*a*), drawing upon data from two closely related dinopids (Blest 1978), points out that the ultrastructural organization of receptive segments can differ radically within small taxa, and that photoreceptors can be supposed to be vulnerable to powerful selection pressures.

5. Discussion

On a broader canvas, the post-embryonic development of a principal eye can be considered in the following terms.

1. The optical apparatus develops through a collaboration between four components: (*a*) the glass cells that make the corneal lens; (*b*) the retinal tube, which is derived from the pigmented hypodermis, and whose length largely determines the distance between the corneal lens and the retina; (*c*) the mosaic organization and axial positions of the four tiers of receptive segments and their rhabdomeres; (*d*) the pigmented glial cells whose distal, non-pigmented processes provide the matrix in which the receptive segments are embedded.

2. At no point during post-embryonic morphogenesis of a principal retina have we seen either mitotic figures, or any evidence for the programmed death of primordial cells (Blest 1988*b*). We assume that the development of a principal retina and of its dioptrics results solely from the differentiation of an initial population of cells, and interactions between them.

3. Tiering of the receptive segments has been shown to result primarily from gross conformational changes to a retina at around days 4–5, the transformation presumably being generated by growth of the glial processes (Blest 1988*b*).

4. Within a principal retina, the ordered transverse spacing between receptive segments and their rhabdomeres must be contrived by interactions between nascent receptive segments and processes of the pigmented glia. Eakin & Brandenburger (1971) first showed that the principal

retinae of salticids contain only one type of glial cell, whose somata lie proximally among those of the receptors. Their pigment granules collaborate to form a screen that protects the retina from stray light transmitted through the prosoma; effectively, the pigmented region forms a cup within which the four tiers of receptive segments lie. The role of the distal glial processes is merely architectural.

5. An optical analysis of the principal retina of *Plexippus* by Blest *et al.* (1981) implied that the level of error in the longitudinal dimensions of the dioptrics cannot exceed some 3% if an eye is to work effectively. Because the retinal tubes of some large species can be some 1.5 mm long (Blest 1985*b*) the degree of developmental regulation is remarkable, given the number of components that must collaborate to ensure it.

How is this quantitatively accurate performance guaranteed? The longitudinal dimensions of the retinal tube and their relation to the shape of the corneal lens cannot usefully be discussed from the present data.

Nor is it immediately obvious how the developmental regulation of a principal retina might relate to what is known about the morphogenesis of arthropod compound eyes (see, for example, Eley & Shelton 1976; Shelton & Lawrence 1974; Hafner *et al.* 1982). There is no equivalent of the 'crystalline' order exhibited by the arrays of ommatidia in compound eyes. Recently, Tomlinson & Ready (1986) have assessed the sequence by which the eight photoreceptors within an ommatidium of *Drosophila* acquire independent identities, by labelling them as they develop with neuron-specific antibodies. The receptors are directed into their developmental pathways by a well defined maturational sequence which is not lineage dependent. Instead, local control of assembly and differentiation results from a combinatorial code from which the fate of a given nascent photoreceptor is determined by signals received from neighbouring cells with which it is in contact.

The mechanical process that establishes tiering in a principal retina suggests that the starting positions of primordial receptive segments are critical for their subsequent fates. Early receptive segments are certainly in direct contact with one another until at least day 7′, so that some part of their subsequent differentiation might be determined by a combinatorial code, as in *Drosophila*. The later outgrowth of the glial processes, however, introduces many difficulties. Micrographs suggest that there are far fewer glial somata than receptive segments. Eakin & Brandenburger (1971) noted that in the accessory eyes of salticids a very small population of distal non-pigmented glial cells (absent from the principal eyes) supplies processes to the field of receptive segments. The processes provided by a single glial soma extend over large populations of receptive segments, but the field served by any one soma is irregular. If the relation between pigmented glial cells and receptive segments in a principal eye is similar, which seems likely, it is difficult to suppose that the fates of receptors after around day 10′ are determined by contact signals derived from the glial processes.

The transverse and longitudinal dimensions of the receptive segments of Layers I and II (2b and c) exhibit simple gradients extending dorsally and ventrally from the fovea (where segments are narrow) to the periphery (where they are wide), the extreme difference in diameters amounting almost to an order of magnitude. Such size gradients could be simply determined by a single diffusible morphogen, of the kind whose existence has frequently been postulated, and an example of which has now been found and partly characterized (Macdonald & Struhl 1986).

Throughout this demanding study we have received magnificent support from George Weston and the staff of the Australian National University Transmission Electron Microscope Unit.

REFERENCES

Blest, A. D. 1978 The rapid synthesis and destruction of photoreceptor membrane by a dinopid spider. *Proc. R. Soc. Lond.* B **200**, 463–483.

Blest, A. D. 1985*a* The fine structure of spider photoreceptors in relation to function. In *The neurobiology of arachnids* (ed. F. G. Barth), pp. 79–102. Berlin, Heidelberg, New York and Tokyo: Springer-Verlag.

Blest, A. D. 1985*b* The retinal mosaics of the principal eyes of some neotropical jumping spiders: optical trade-offs between sizes and habitat illuminances. *J. comp. Physiol.* A **157**, 391–404.

Blest, A. D. 1987 Comparative aspects of the retinal mosaics of jumping spiders. In *Arthropod brain: its evolution, development, structure and function* (ed. A. P. Gupta), pp. 203–229. New York: Wiley.

Blest, A. D. 1988*a* The turnover of phototransductive membrane in compound eyes and ocelli. *Adv. Insect Physiol.* **20**, 1–53.

Blest, A. D. 1988*b* Post-embryonic development of the principal retina of a jumping spider. I. The establishment of receptor tiering by conformational changes. *Phil. Trans. R. Soc. Lond.* B **320**, 489–504. (Preceding paper.)

Blest, A. D. & Day, W. A. 1978 The rhabdomere organization of some nocturnal pisaurid spiders in light and darkness. *Phil. Trans. R. Soc. Lond.* B **283**, 1–23.

Blest, A. D., De Couet, H. G., Howard, J., Wilcox, M. & Sigmund, Claudia 1984 The extrarhabdomeral cytoskeleton in photoreceptors of Diptera. I. Labile components in the cytoplasm. *Proc. R. Soc. Lond.* B **220**, 339–352.

Blest, A. D., Hardie, R. C., McIntyre, P. & Williams, D. S. 1981 The spectral sensitivities of identified receptors and the function of retinal tiering in the principal eye of a jumping spider. *J. comp. Physiol.* A **145**, 227–239.

Blest, A. D. & Maples, Joanne 1979 Exocytotic shedding and glial uptake of photoreceptor membrane by a salticid spider. *Proc. R. Soc. Lond.* B **204**, 105–112.

Blest, A. D., McIntyre, P. & Carter, M. 1988 A re-examination of the principal retinae of *Phidippus johnsoni* and *Plexippus validus* (Araneae: Salticidae): implications for optical modelling. *J. comp. Physiol.* A **162**, 47–56.

Blest, A. D. & Price, G. D. 1984 Retinal mosaics of the principal eyes of some jumping spiders (Salticidae: Araneae): adaptations for high visual acuity. *Protoplasma* **120**, 172–184.

Blest, A. D. & Sigmund, Claudia 1984 Retinal mosaics of the principal eyes of two primitive jumping spiders. *Yaginumanis* and *Lyssomanes*: clues to the evolution of Salticid vision. *Proc. R. Soc. Lond.* B **221**, 111–125.

Blest, A. D. & Sigmund, C. 1985*a* Retinal mosaics of a primitive jumping spider, *Spartaeus* (Araneae, Salticidae, Spartaeinae): a phylogenetic transition between low and high visual acuities. *Protoplasma* **125**, 129–139.

Blest, A. D. & Sigmund, C. 1985*b* The cytoskeletal architecture of the inter-digitated photoreceptor microvilli of some nocturnal spiders. *Protoplasma* **125**, 153–161.

Blest, A. D., Stowe, Sally, Eddey, Wendy & Williams, D. S. 1982 The local deletion of a microvillar cytoskeleton from photoreceptors of tipulid flies during membrane turnover. *Proc. R. Soc. Lond.* B **215**, 469–479.

Blest, A. D., Williams, D. S. & Kao, L. 1980 The posterior median eyes of the dinopid spider *Menneus*. *Cell Tissue Res.* **211**, 391–403.

Eakin, R. M. & Brandenburger, J. 1971 Fine structure of the eyes of jumping spiders. *J. Ultrastruct. Res.* **37**, 618–663.

Eley, S. & Shelton, P. M. J. 1976 Cell junctions in the developing compound eye of the desert locust *Schistocerca gregaria*. *J. Embryol. exp. Morph.* **36**, 409–423.

Hafner, G. S., Tokarski, T. R. & Hammond-Soltis, G. 1982 Development of the crayfish retina: a light and electron microscope study. *J. Morphol.* **173**, 101–108.

Land, M. F. 1969 Structure of the retinae of jumping spiders (Salticidae: Dendryphantinae) in relation to visual optics. *J. exp. Biol.* **51**, 443–470.

Macdonald, P. M. & Struhl, G. 1986 A molecular gradient in early *Drosophila* embryos and its role in specifying the body pattern. *Nature, Lond.* **324**, 537–545.

Shelton, P. M. J. & Lawrence, P. A. 1974 Structure and development of ommatidia in *Oncopeltus fasciatus*. *J. Embryol. exp. Morphol.* **32**, 337–353.

Snyder, A. W. 1979 The physics of vision in compound eyes. In *Handbook of sensory physiology* VII/6A (ed. H. Autrum), pp. 225–313. Berlin, Heidelberg and New York: Springer-Verlag.

Tomlinson, A. & Ready, D. F. 1986 *Drosophila* photoreceptors express neural antigens in a precise sequence. In *Abstracts from molecular neurobiology of Drosophila*, p. 14. New York: Cold Spring Harbor Laboratory.

Uehara, A., Toh, Y. & Tateda, H. 1977 Fine structure of the eyes of orb-weavers, *Argiope amoena* L. Koch (Araneae: Argyopidae). 1. The antero-medial eyes. *Cell Tiss. Res.* **182**, 81–91.

Uehara, A., Toh, Y. & Tateda, H. 1978 Fine structure of the eyes of orb-weavers, *Argiope amoena* L. Koch (Araneae: Argyopidae). 2. The antero-lateral, postero-lateral and postero-medial eyes. *Cell Tiss. Res.* **186**, 435–452.

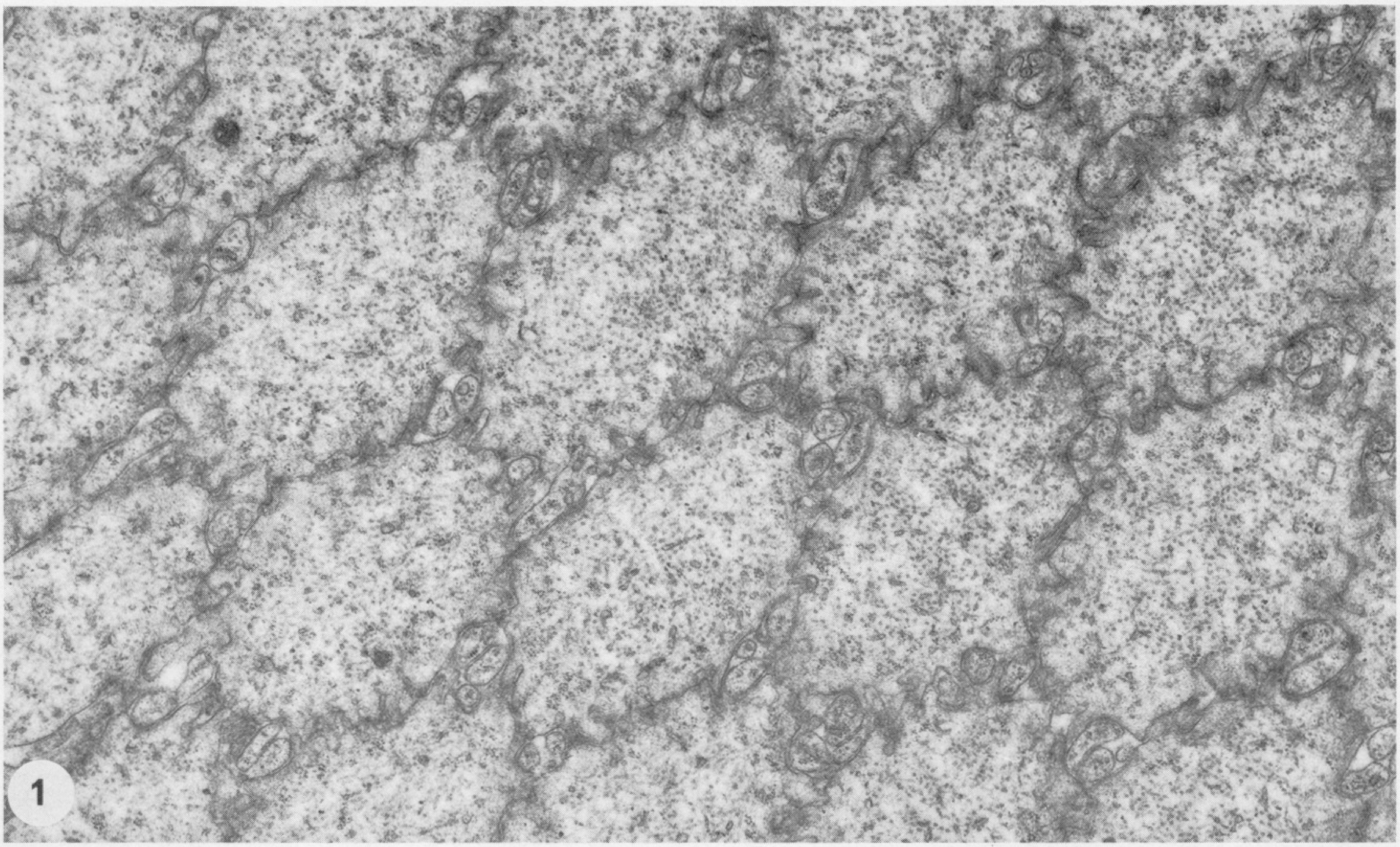
Williams, D. S. & McIntyre, P. 1980 The principal eyes of a jumping spider have a telephoto component. *Nature, Lond.* **288**, 578–580.

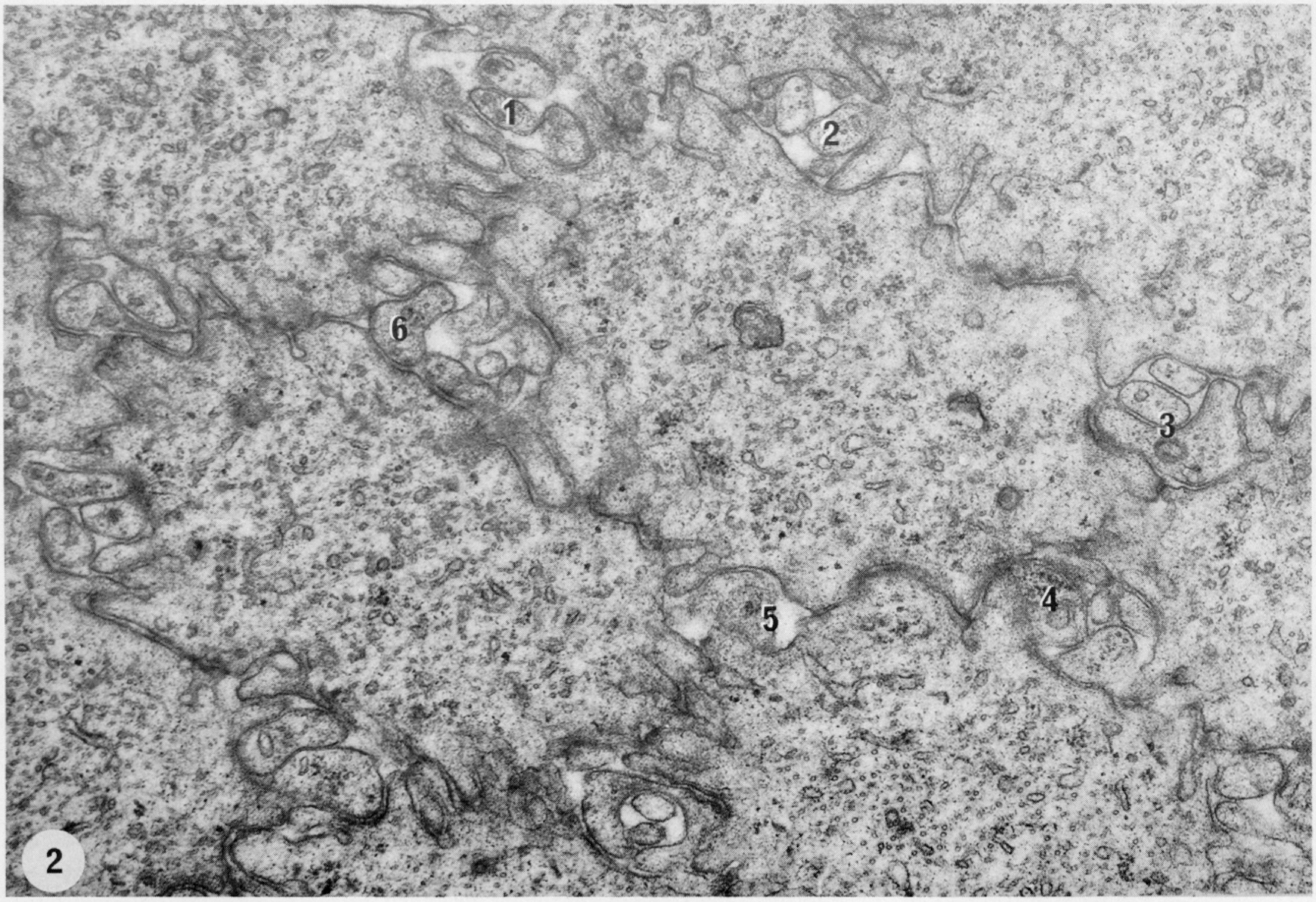
Abbreviations used on the figures

The inner (medial) and outer (lateral) sides of a retina are labelled thus. The approximately horizontal midline of a retina is indicated by large arrows.

Fields of nascent receptive segments viewed in transverse section are labelled in terms of the convention defined by Blest (1988*b*, table 1). For example, Layer III contain 3a and 3b receptive segments, those of 'a' deriving from somata at the outer side of a retina, 'b' from the inner side.

DIST	distal
PROX	proximal
GL.C	glial column
Nu	nucleus
OLM	outer limiting membrane
REC	receptive segment





FIGURES 1 AND 2. For description see opposite.

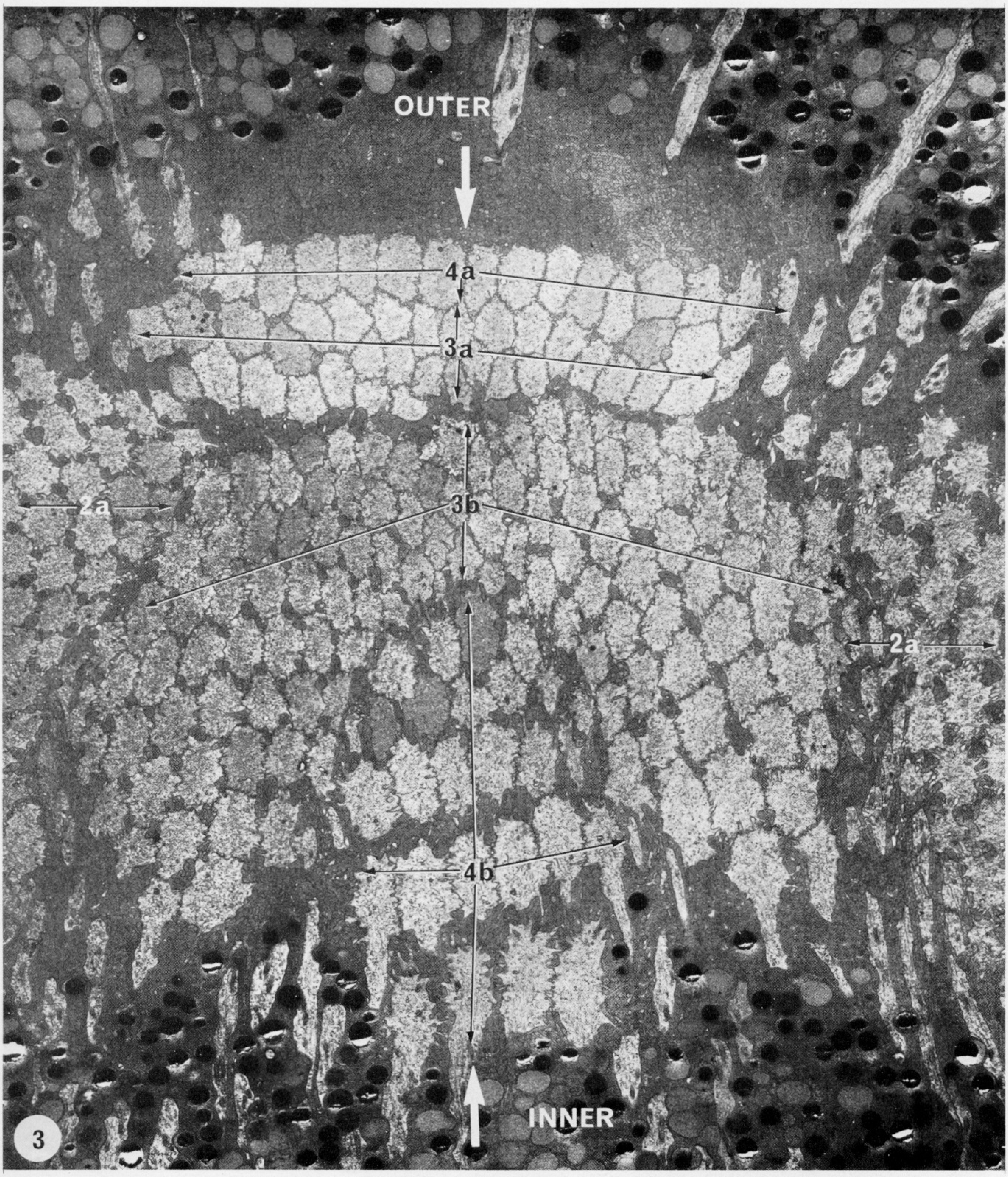


FIGURE 3. A transverse section of a principal retina at day 7′, taken just posterior to the apex of the developing 'pit'. Future receptive segments can be identified by their mosaic configurations and relative positions; their fields are labelled. Around the future optical axis, the outer side of the retina exhibits primordial Layer IV (4 a) receptive segments. Adjacent to them, towards the inner side, are primordial 3 a segments. The mid-region of the field is occupied by primordial 3 b segments, and flanking them, on the inner side, primordial 4 b segments. Ultimately, by day 10′, a single dorsoventral strip of 4 a segments will be juxtaposed to the field of 4 b segments (see figure 13). Peripherally, at day 7′, the foveal field illustrated is flanked dorsally and ventrally by primordial Layer II (2 c) receptive segments. (Magn. × 3750.)

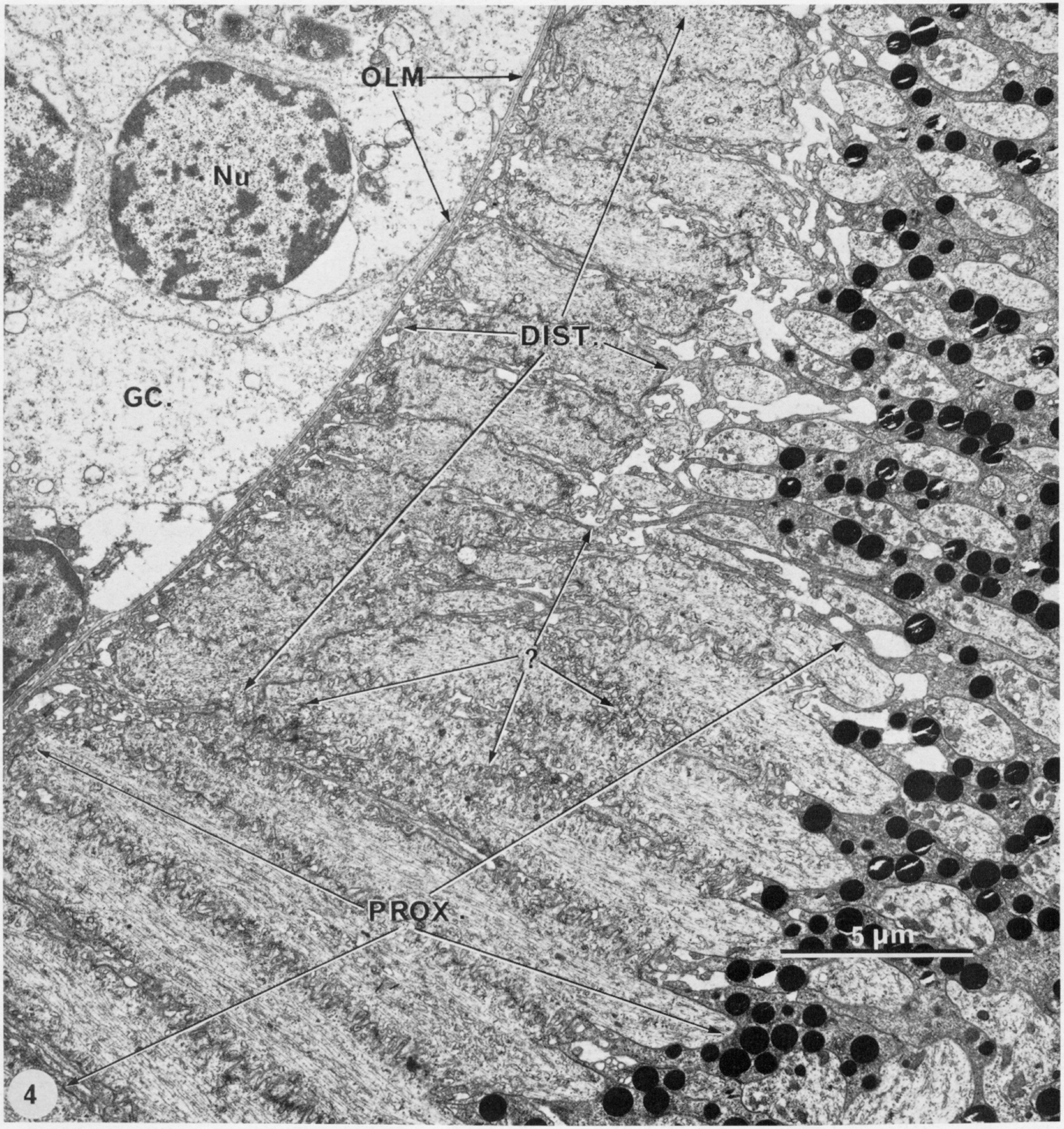


FIGURE 4. A sagittal section of a retina at day 7′. Primordial receptive segments are distinguished as proximal or distal with respect to future optical axis. It is evident that some degree of tiering has already been established. A region of primordial receptive segments is delineated as '?' because it is impossible to attribute them to a specific future Layer. (Magn. ×6250.)

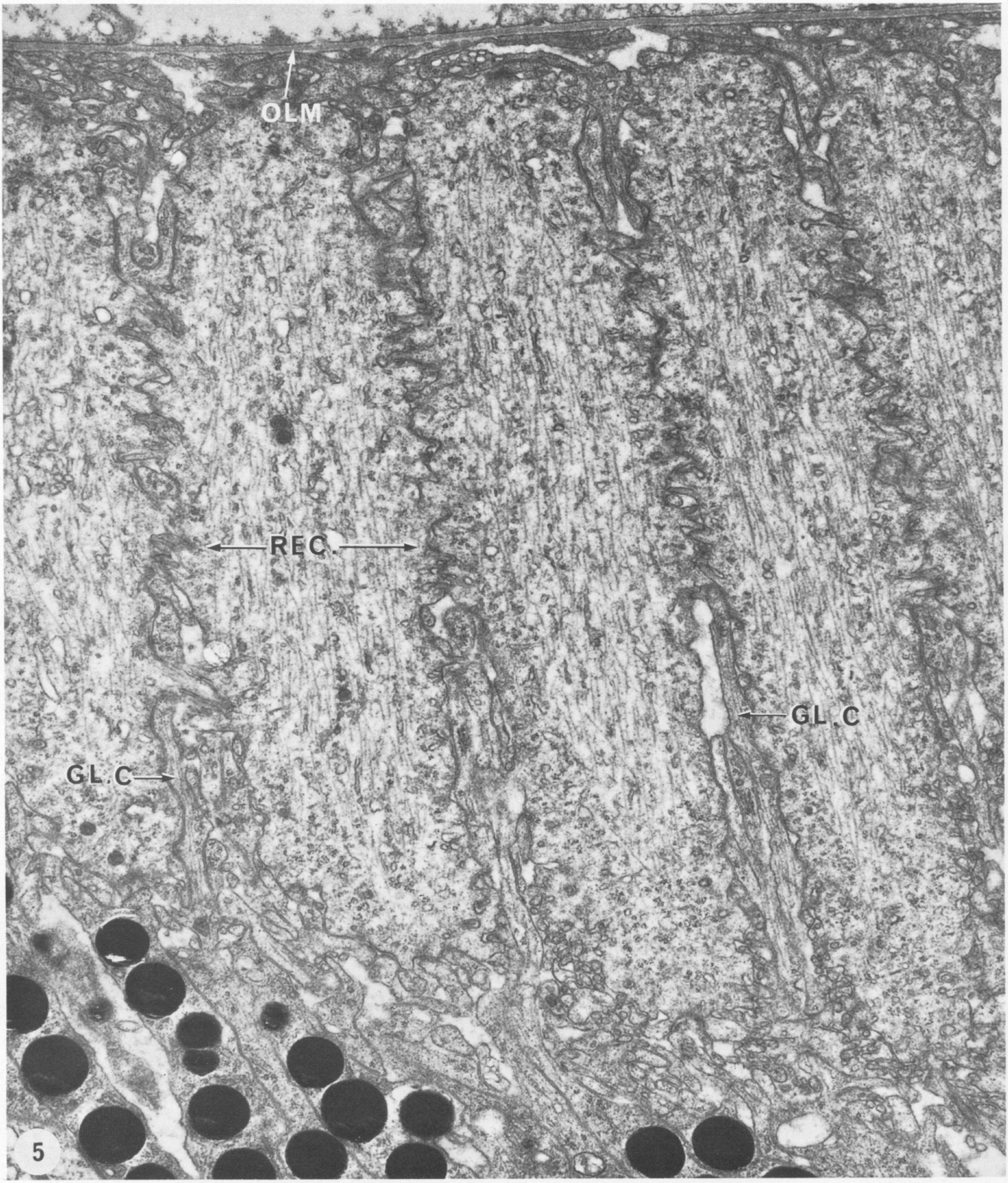
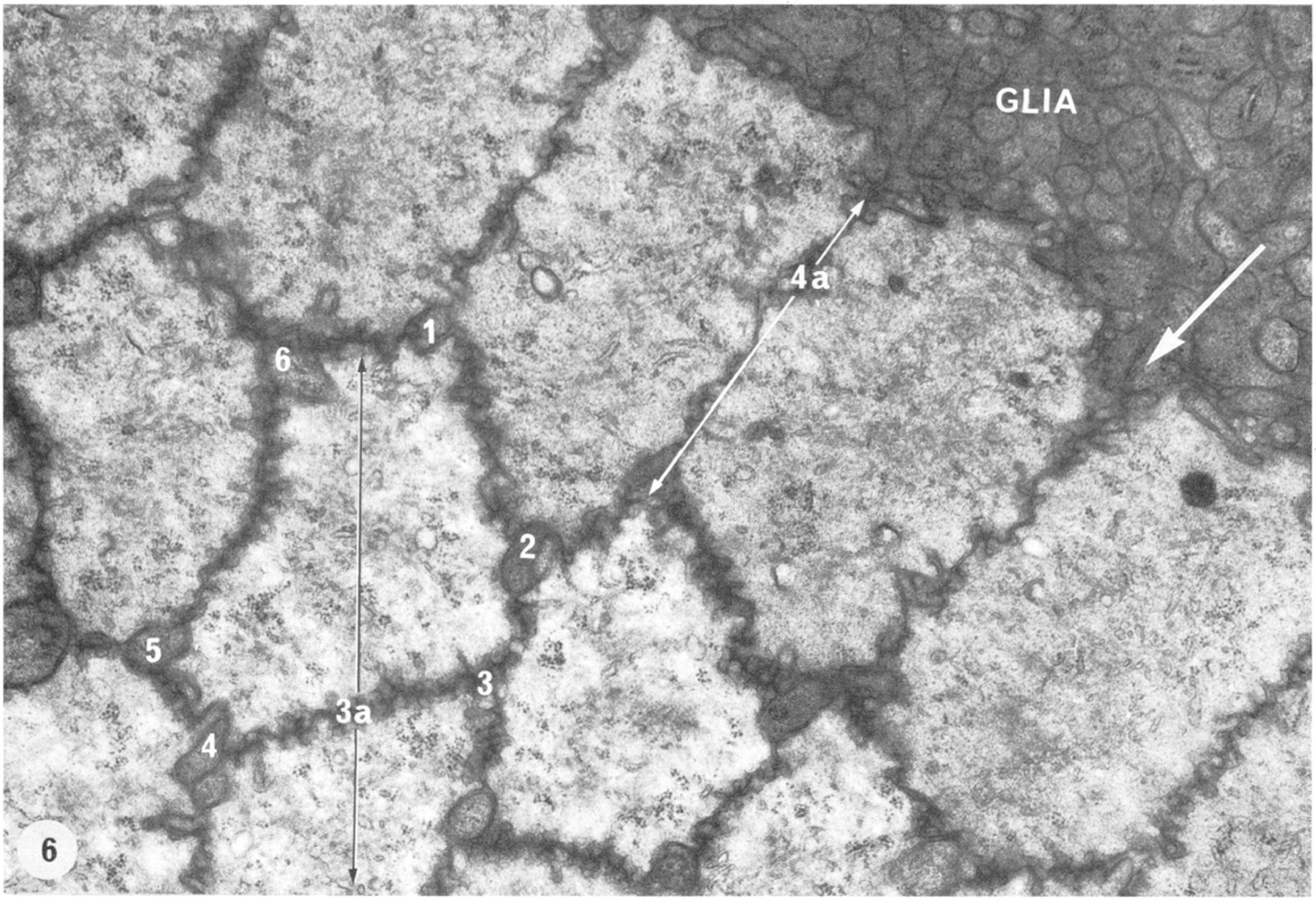
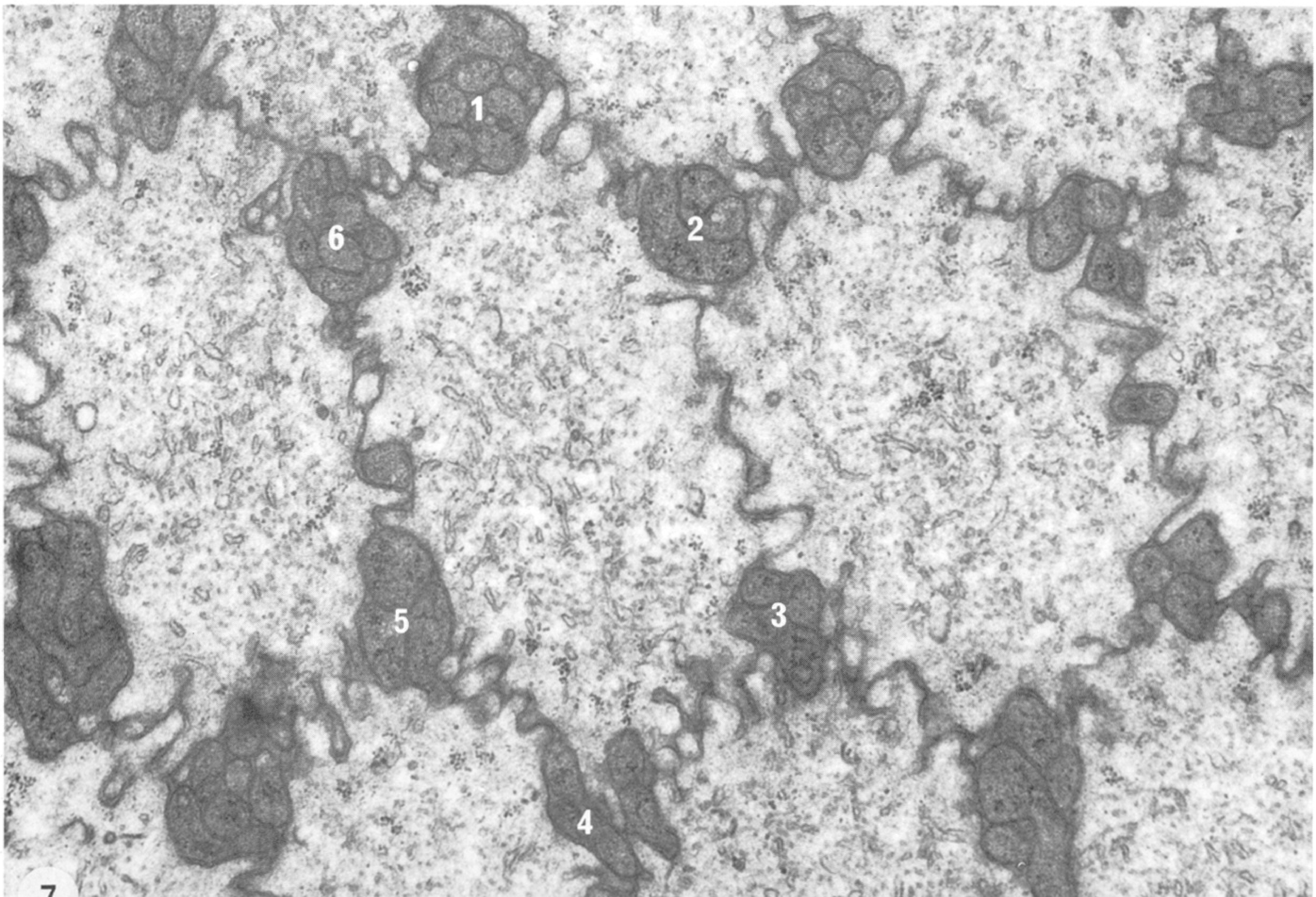
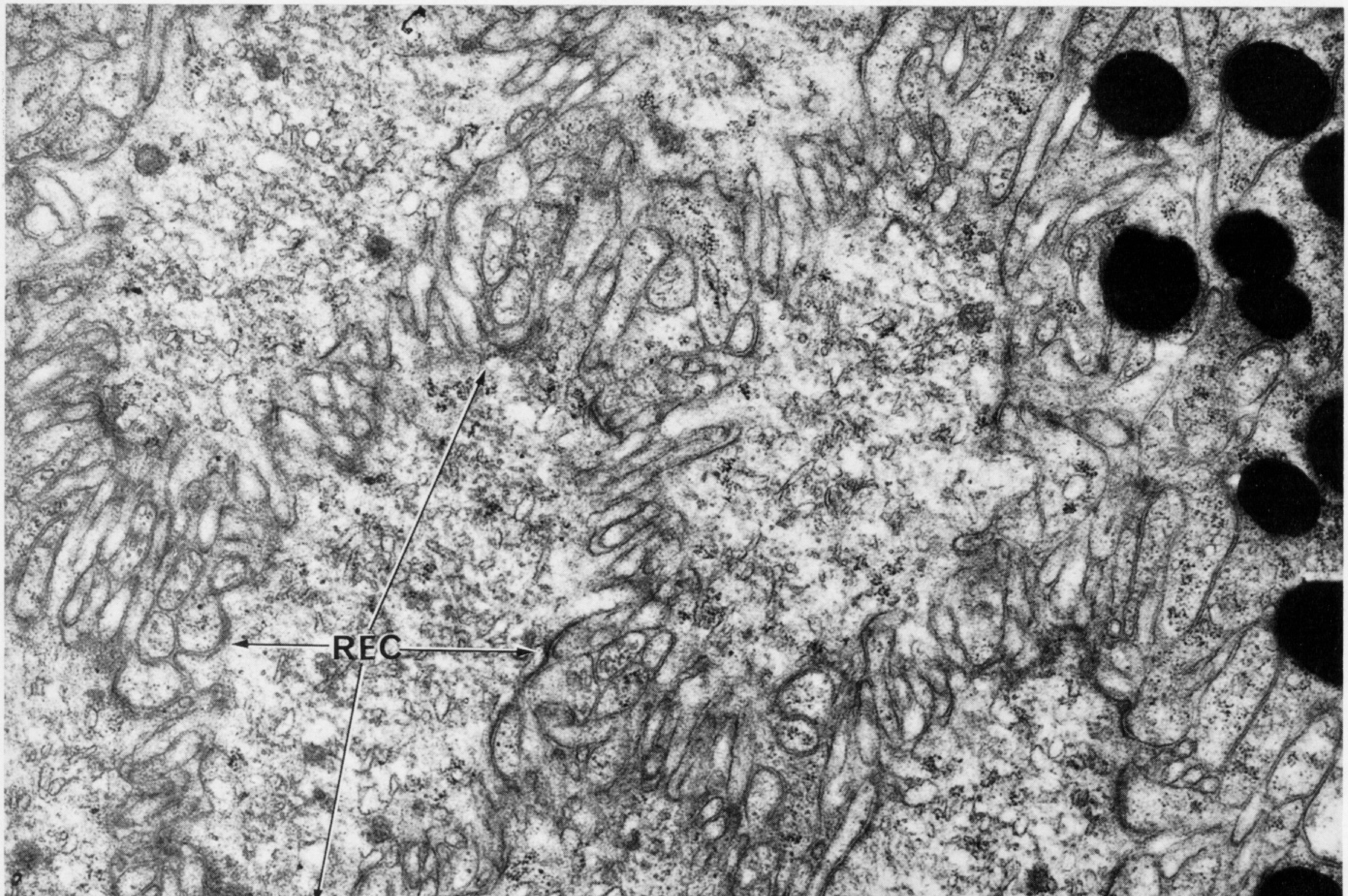


FIGURE 5. A sagittal section of primordial receptive segments near to the future optical axis, at day 7′. (Magn. × 15000.)





FIGURES 6 AND 7. For description see facing plate 6.



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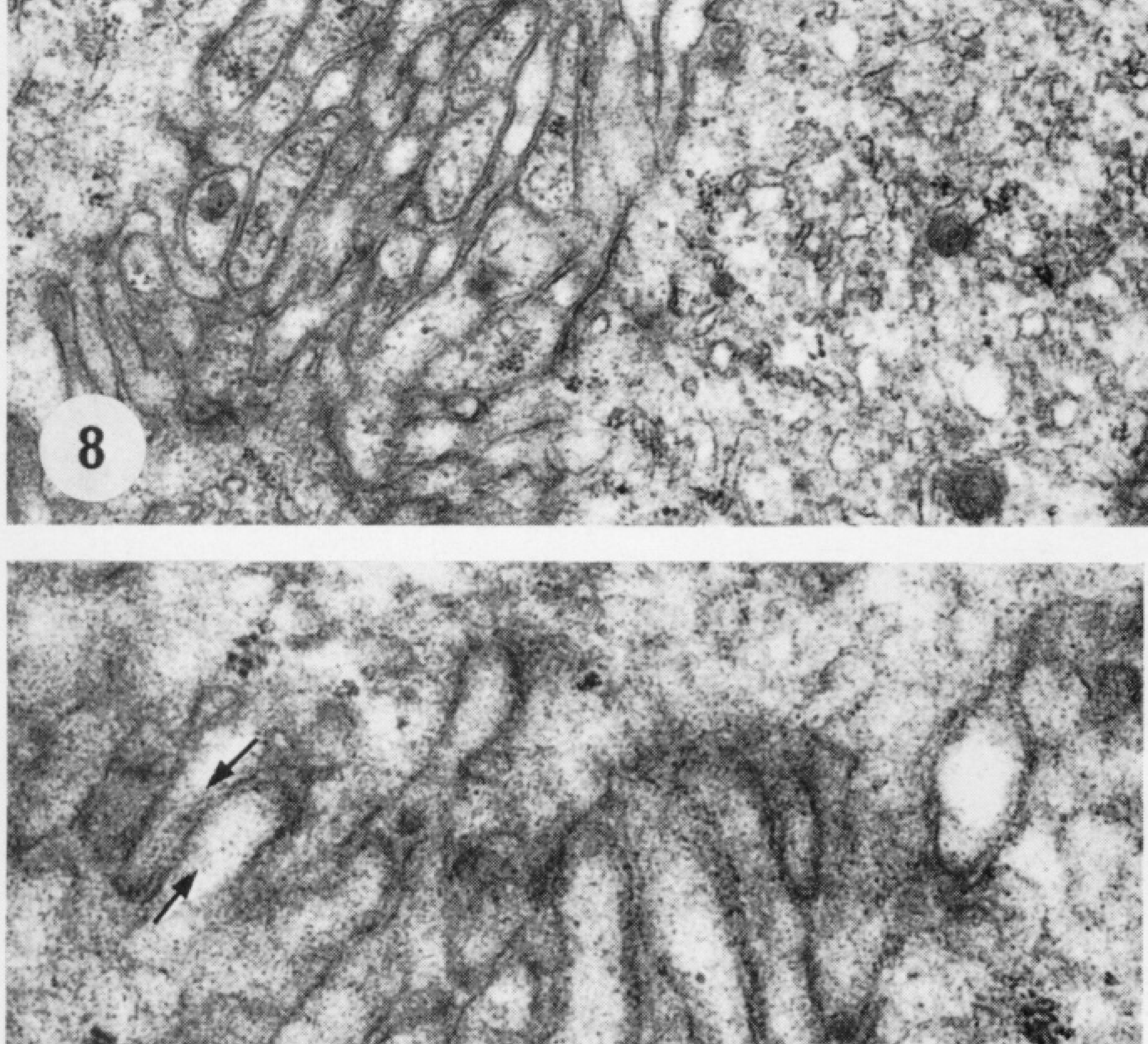

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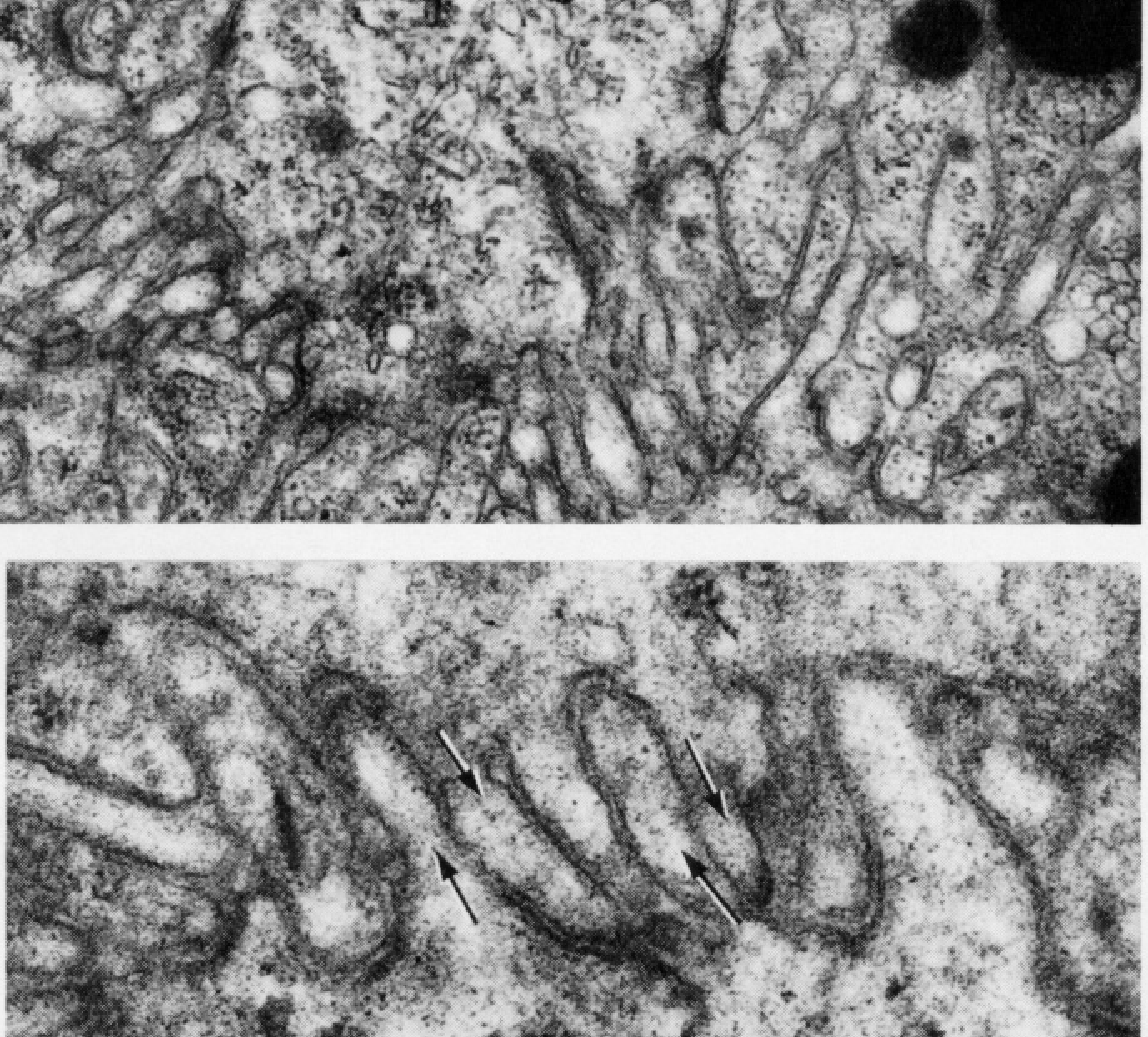

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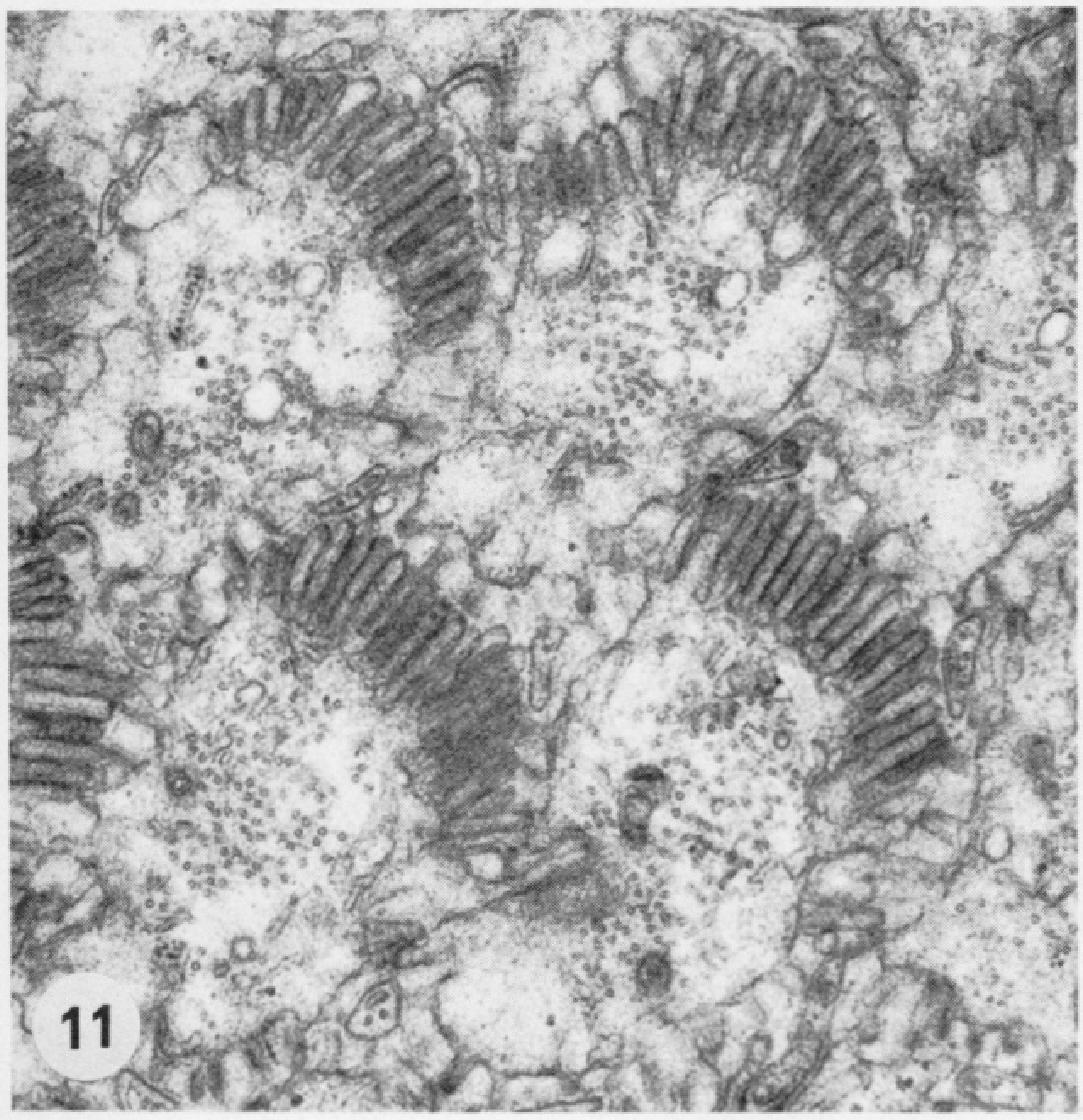
FIGURES 8–10. For description see opposite.



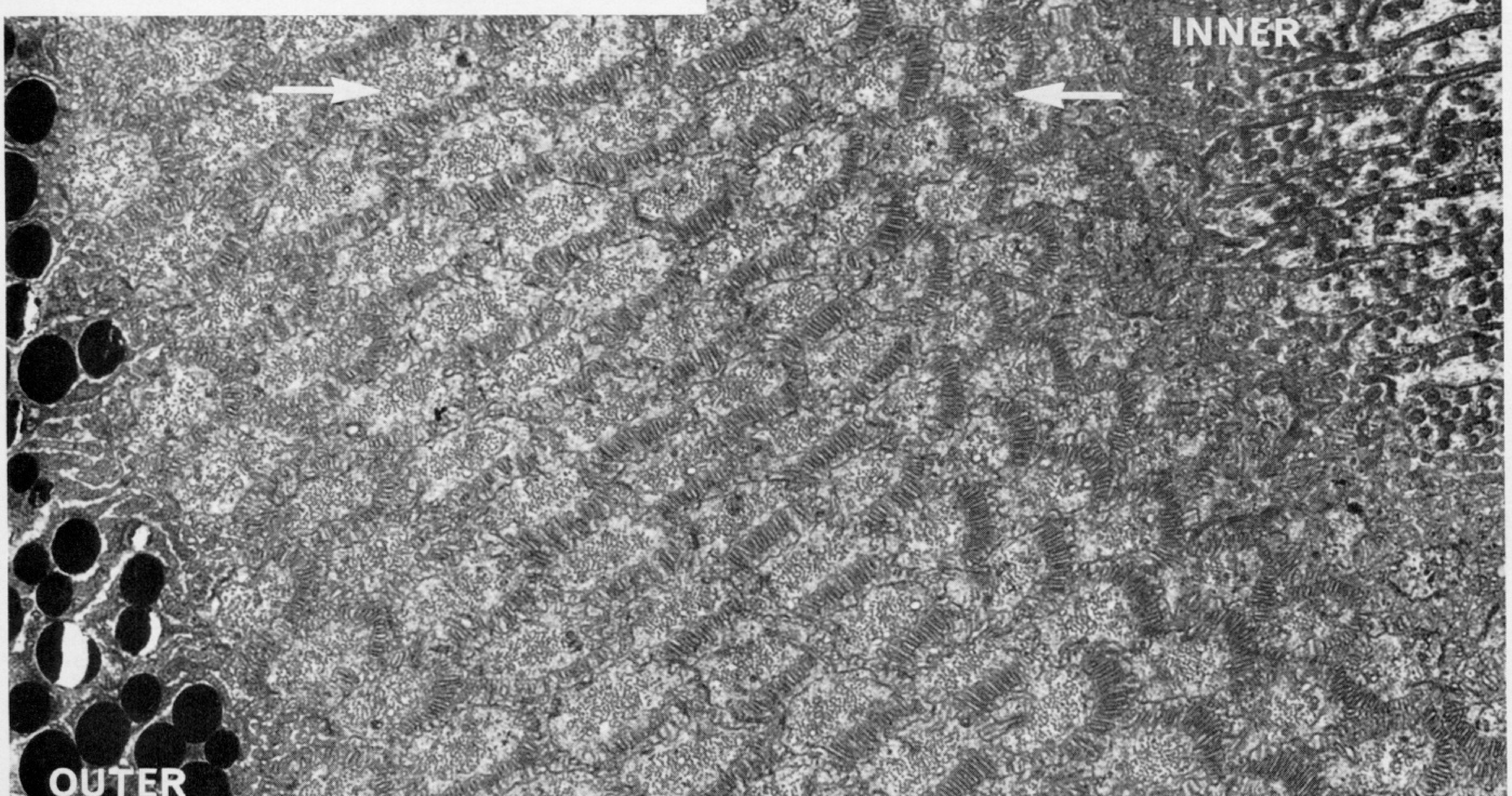
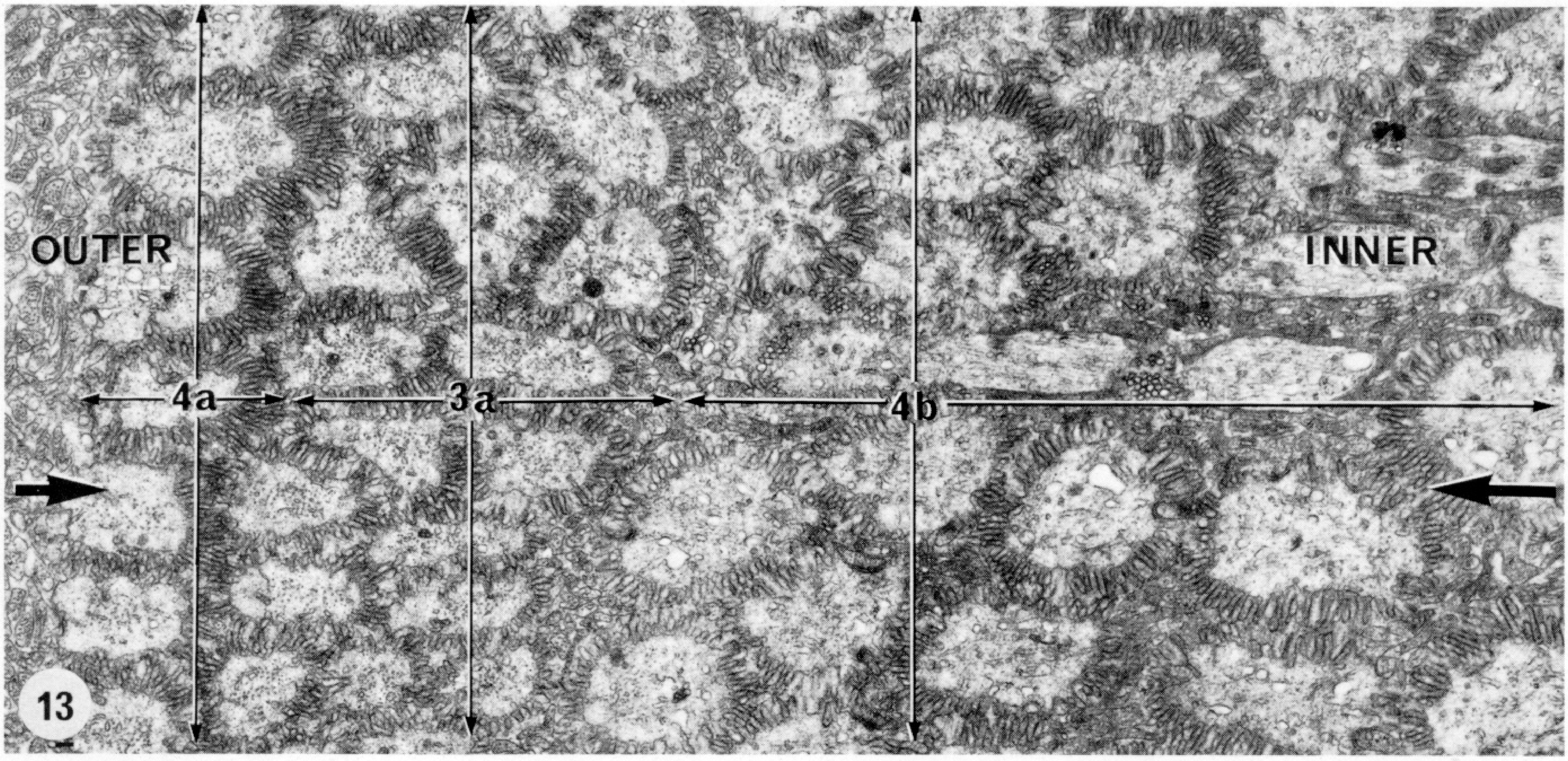


Figure 11. Transverse section of four Layer I receptive segments near to the future optical axis at day 10′. (Magn. × 25000.)

Figure 12. Low-magnification survey of the Layer I mosaic around the future fovea. (Magn. × 7500.)



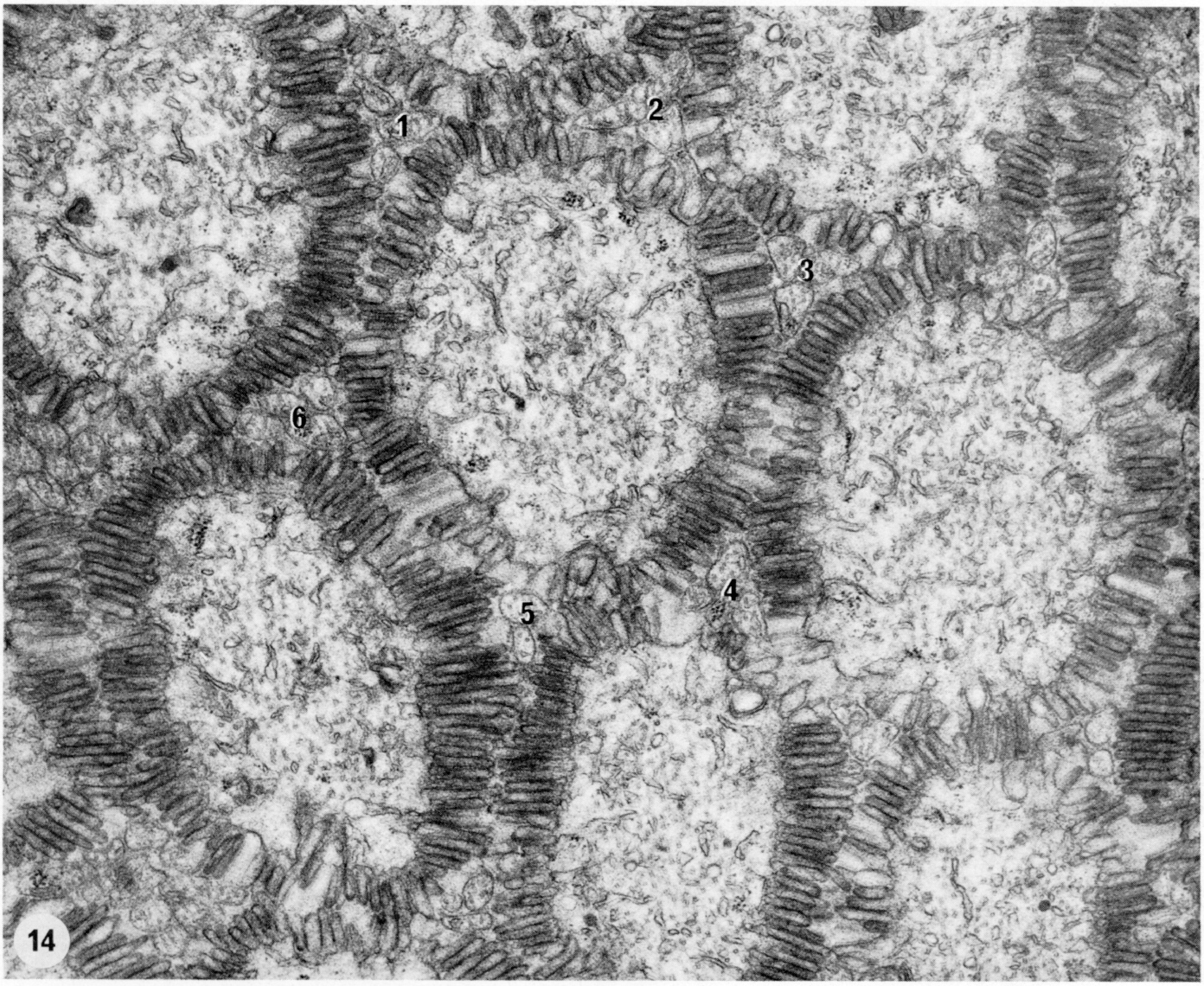


FIGURE 13. Transverse section of Layer IV (4a and 4b) and Layer III (3a) receptive segments, near to the fovea at day 10′. The plane of section is identical to that in figure 3. (Magn. × 10500.)

FIGURE 14. Transverse section through either Layer I or Layer II (2c) receptive segments in the peripheral ventral arm of a retina, at day 10′. (Magn. × 25000.)

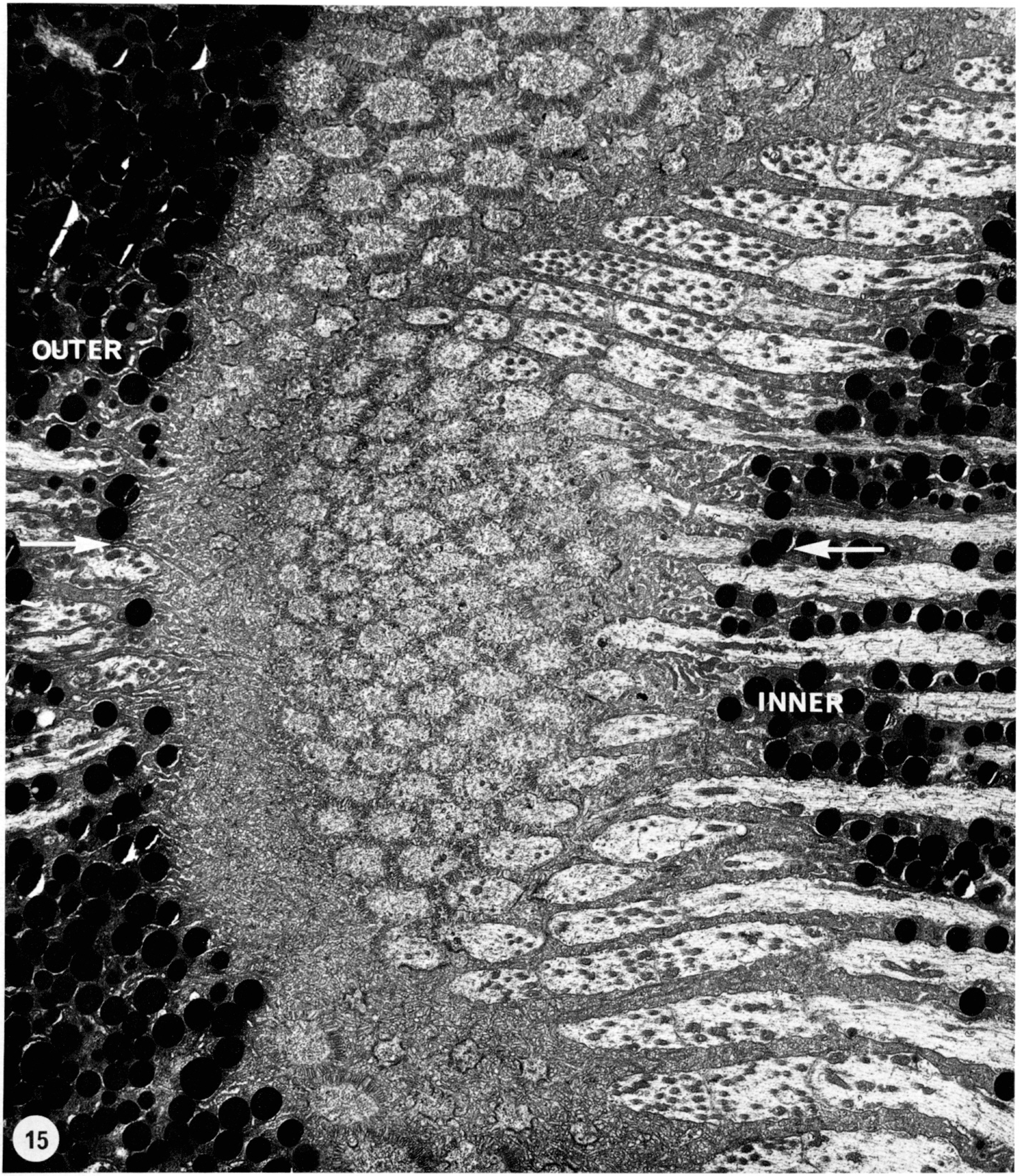
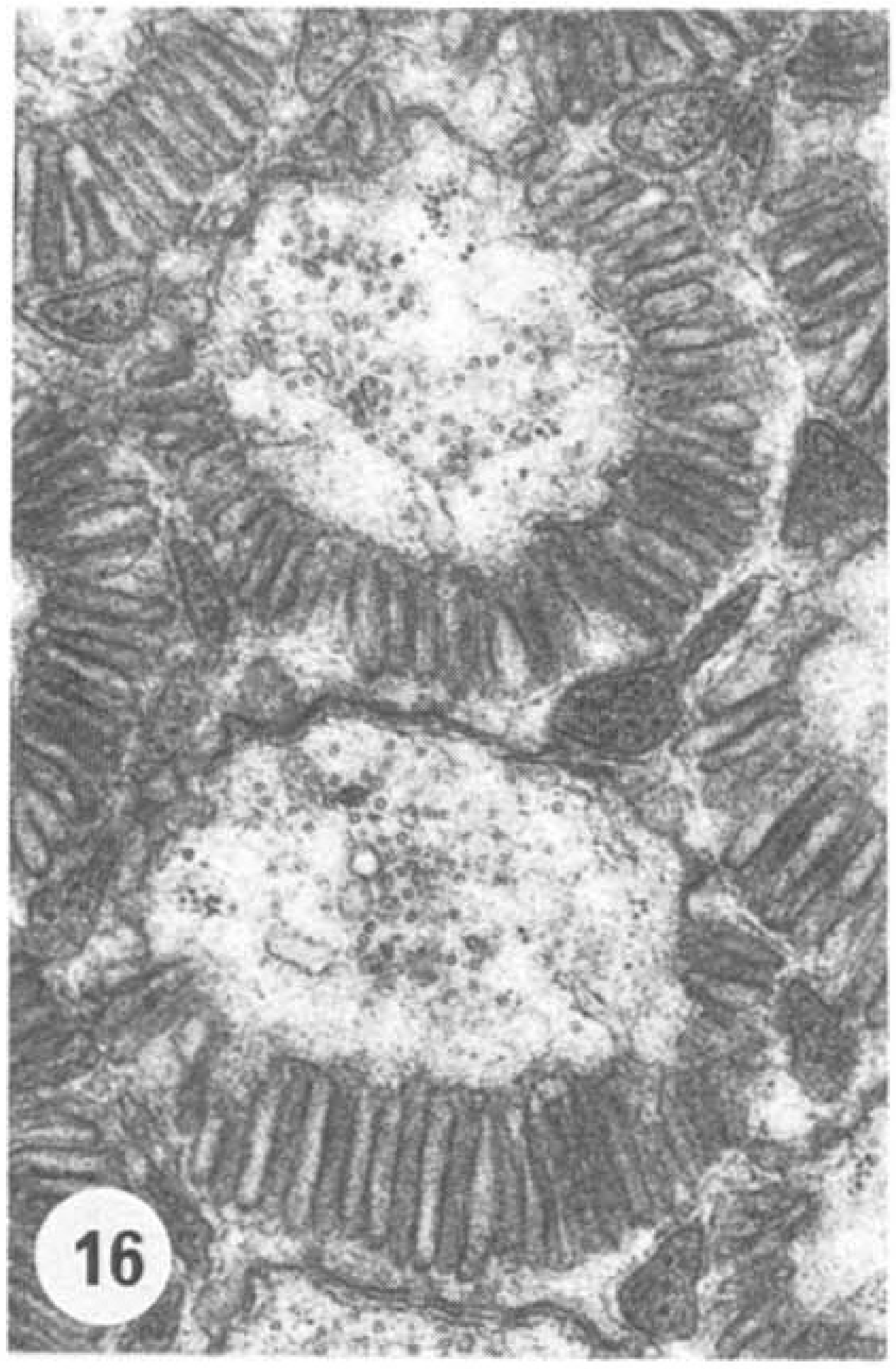


FIGURE 15. Low-magnification survey of the Layer II (2b) receptive segments mosaic, in transverse section, at day 10′. (Magn. × 5000.)



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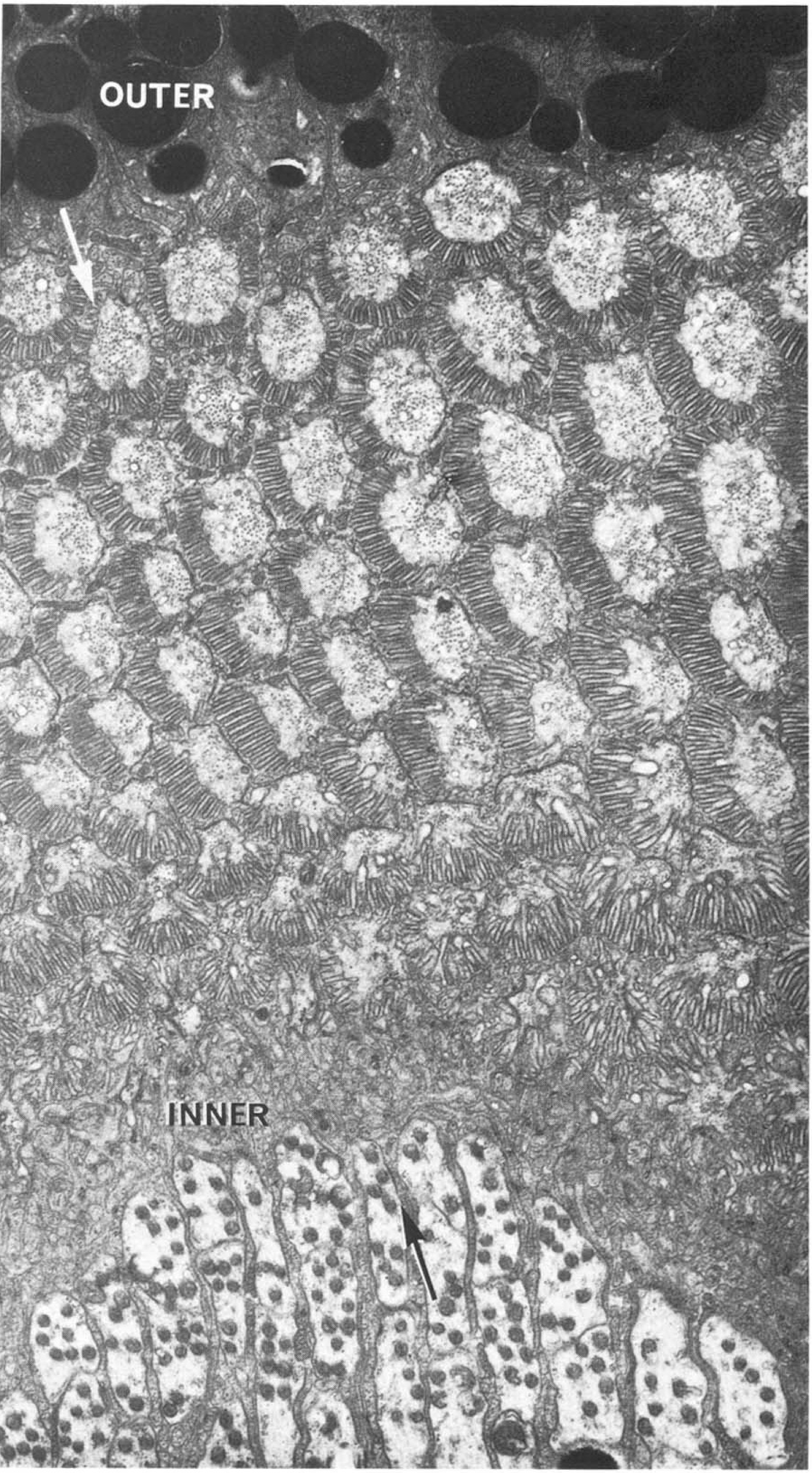

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FIGURE 16. Transverse section of two Layer I receptive segments near to the fovea at day 12. (Magn. × 25000.)

FIGURE 17. Low-magnification survey micrograph of foveal Layer I receptive segments in transverse section, at day 12. (Magn. × 10000.)

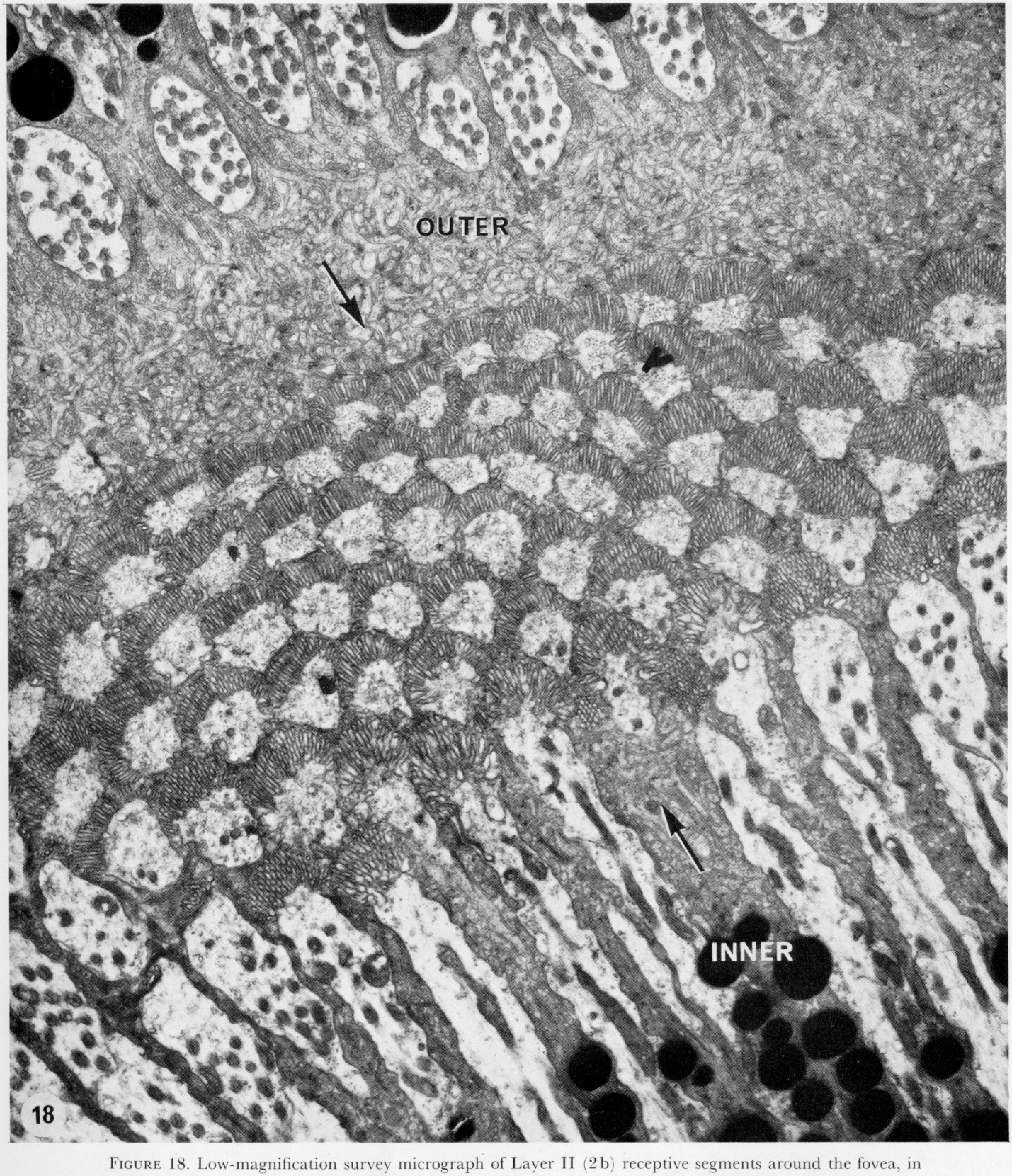

FIGURE 18. Low-magnification survey micrograph of Layer II (2b) receptive segments around the fovea, in transverse section, at day 12. (Magn. × 10000.)

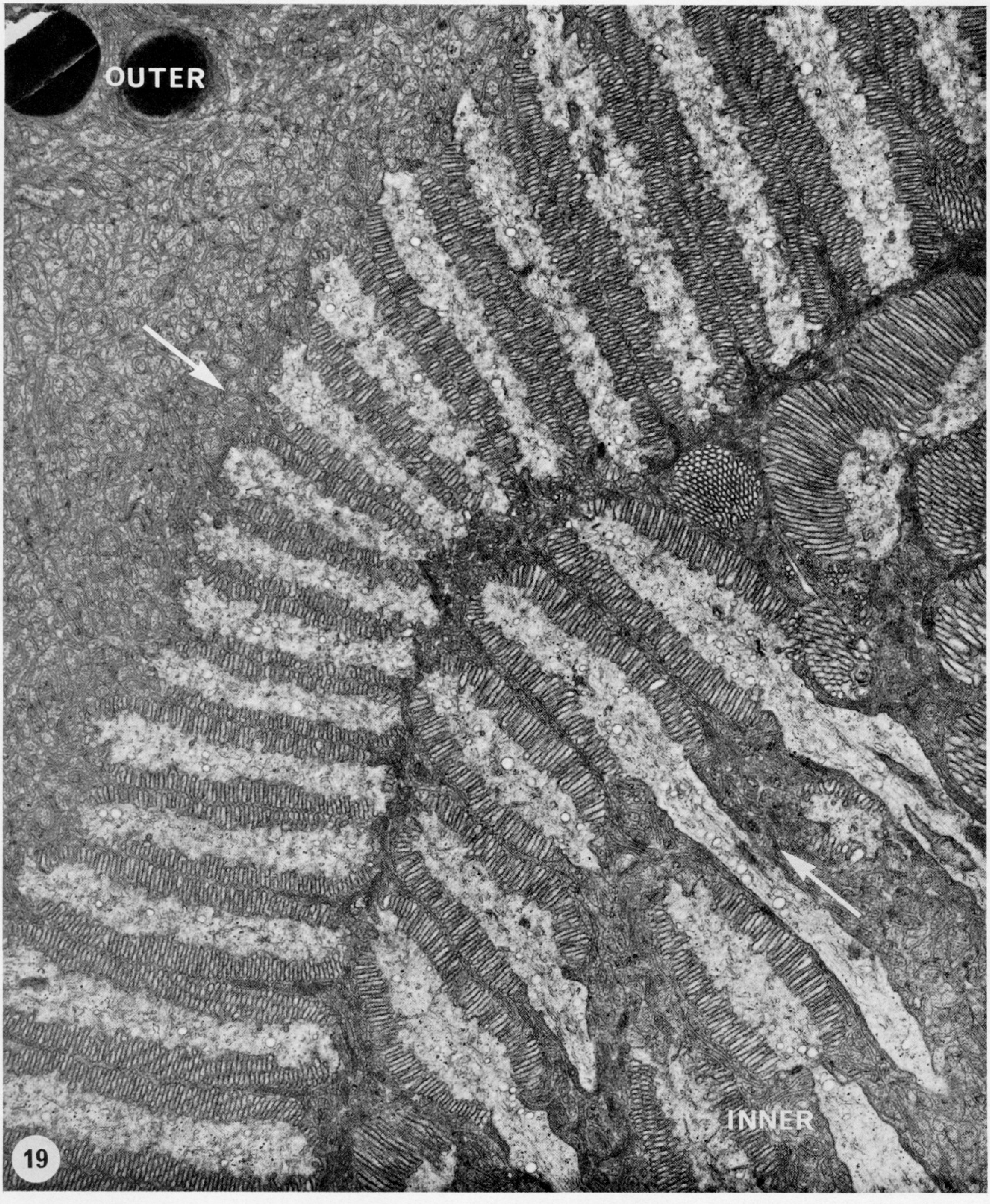

FIGURE 19. Low-magnification micrograph of Layer IV (4a and 4b) receptive segments around the fovea, in transverse section, at day 12. (Magn. ×12500.)

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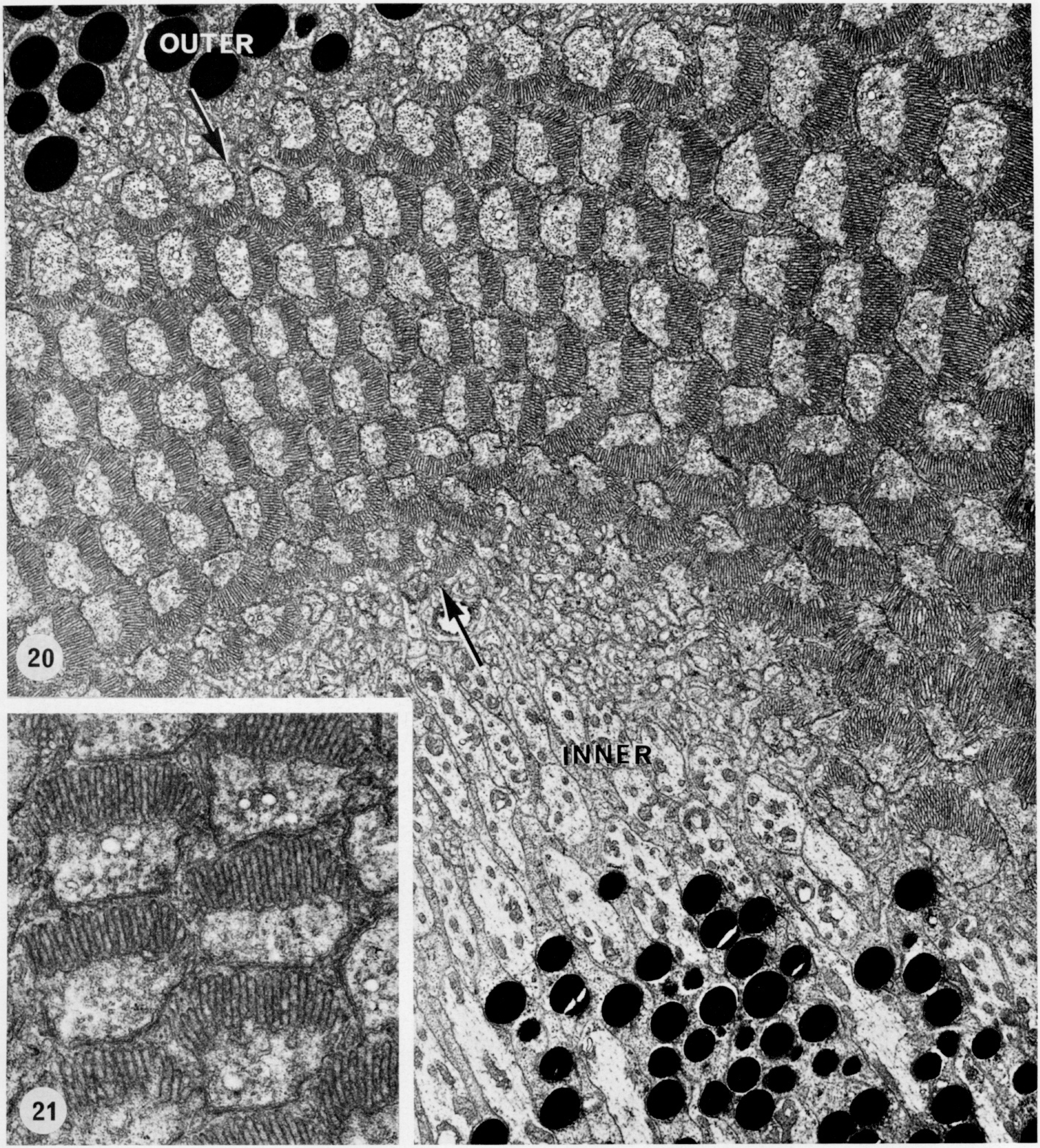
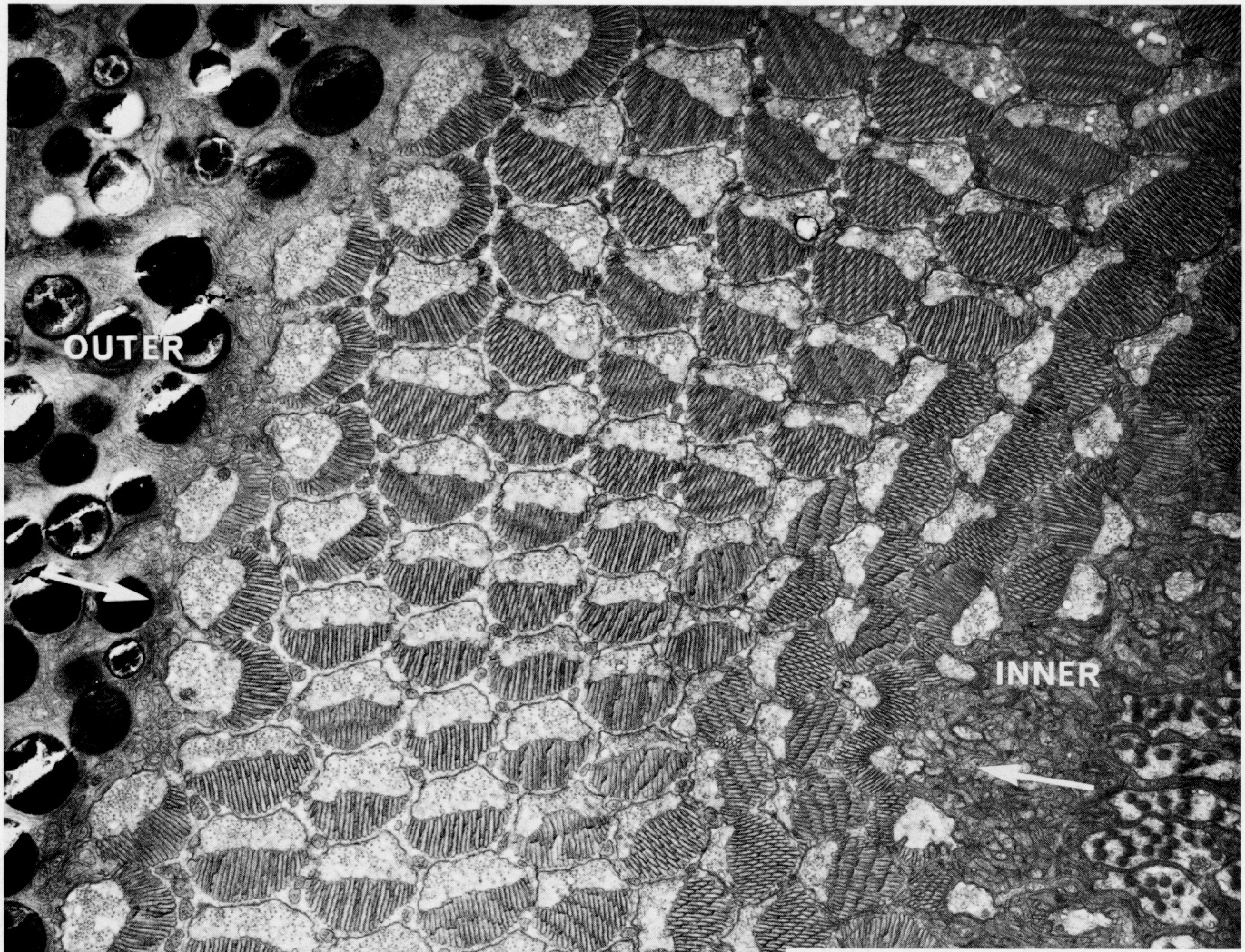


FIGURE 20. Transverse section of Layer I receptive segments around the fovea, at day 13. (Magn. × 8750.)

FIGURE 21. Transverse section of Layer I receptive segments near to the fovea at higher magnification. (Magn. × 25000.)



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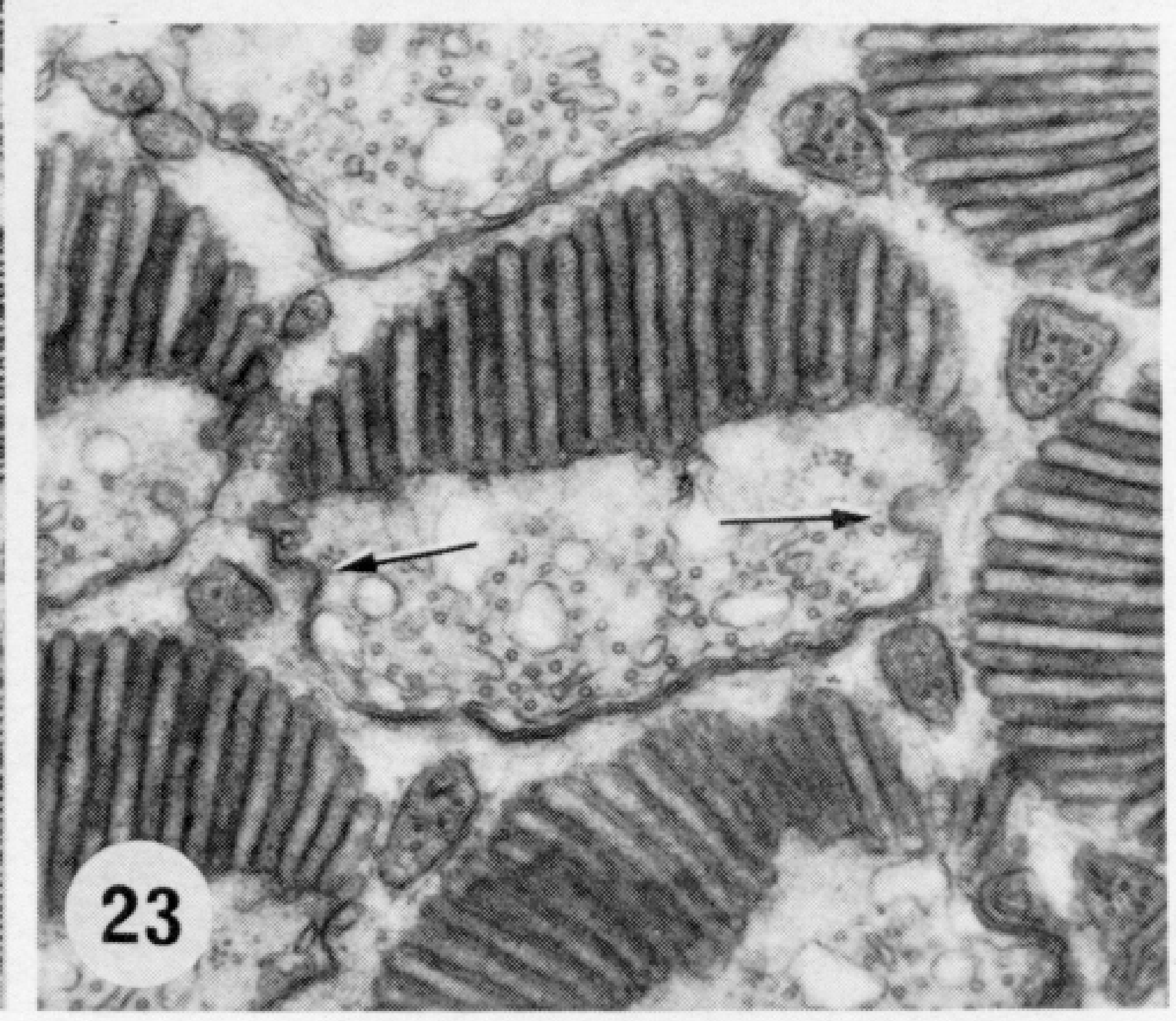

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FIGURE 22. Low-magnification survey micrograph of Layer I receptive segments near to the fovea, in transverse section, at day 14. (Magn. × 10500.)

FIGURE 23. Transverse section of a single Layer I receptive segment at day 14. Two endocytotic pits are indicated by small arrows. (Magn. × 25000.)

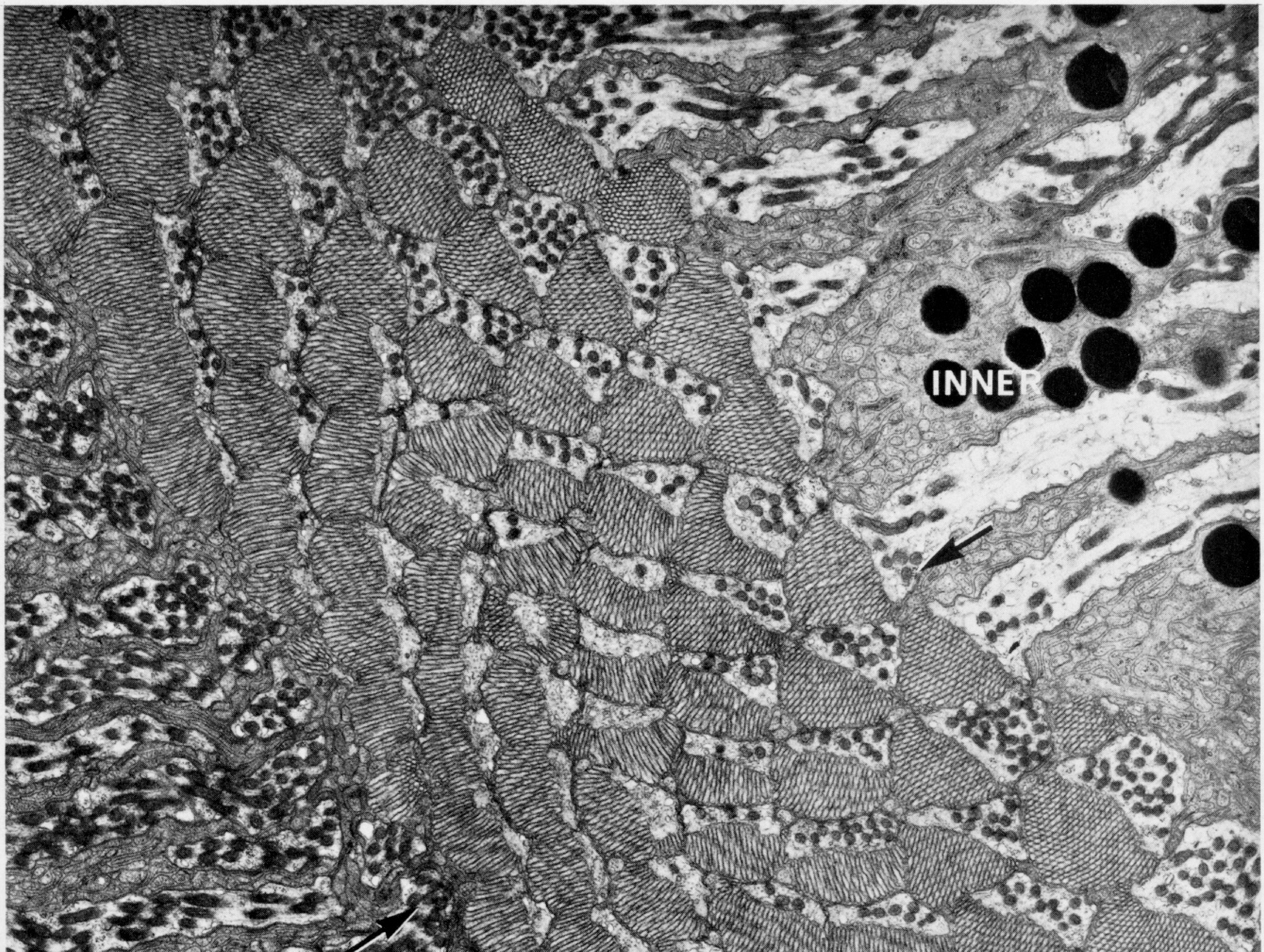

FIGURE 24. Low-magnification survey of Layer II (2b) receptive segments around the fovea, in transverse section, at day 14. (Magn. ×10000.)

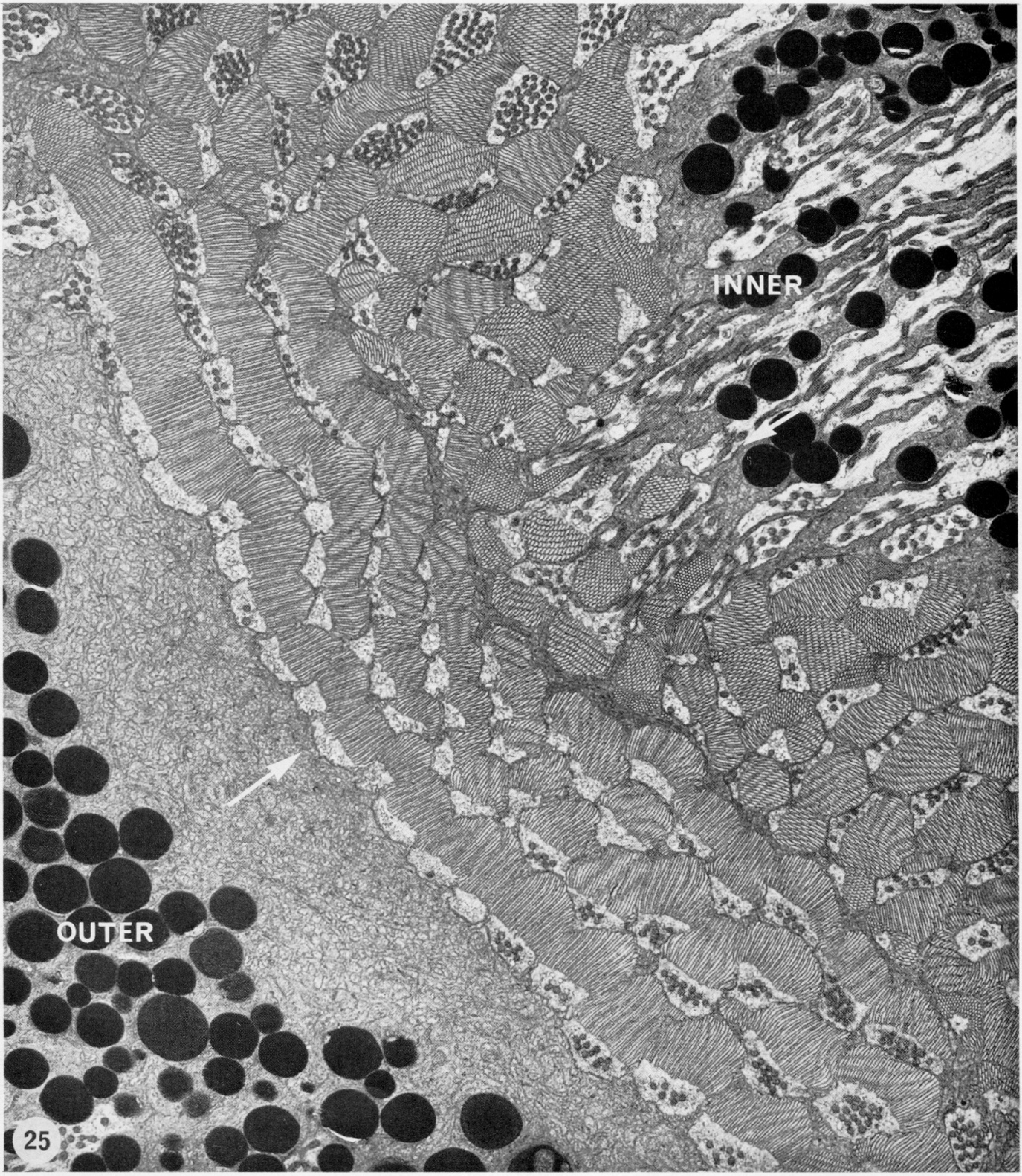

FIGURE 25. Low-magnification survey of Layer III (3a and 3b) receptive segments around the fovea, in transverse section, at day 14. (Magn. × 6150.)

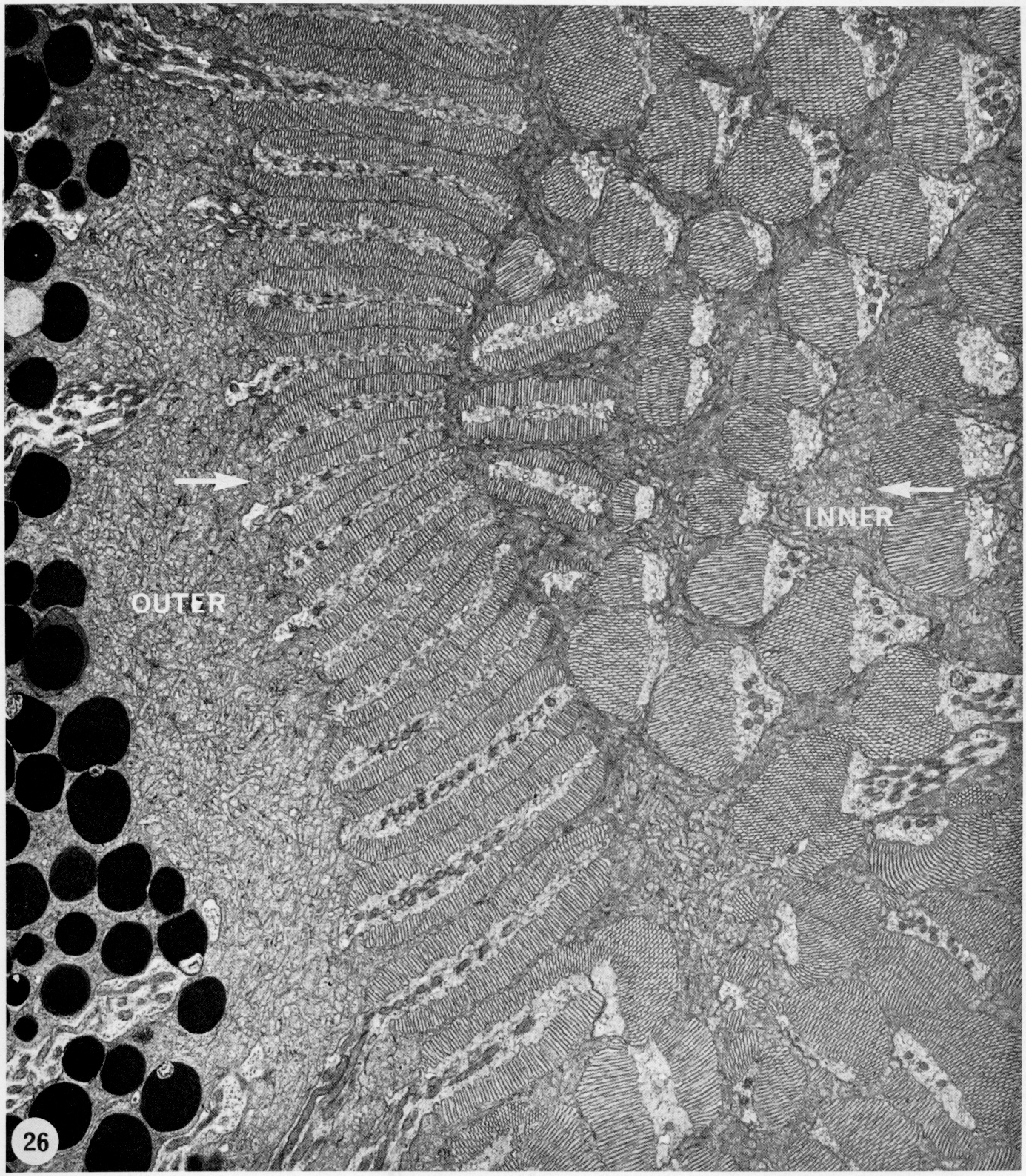

Figure 26. Low-magnification survey of Layer IV (4a and 4b) receptive segments around the fovea, in transverse section, at day 14. (Magn. × 8750.)

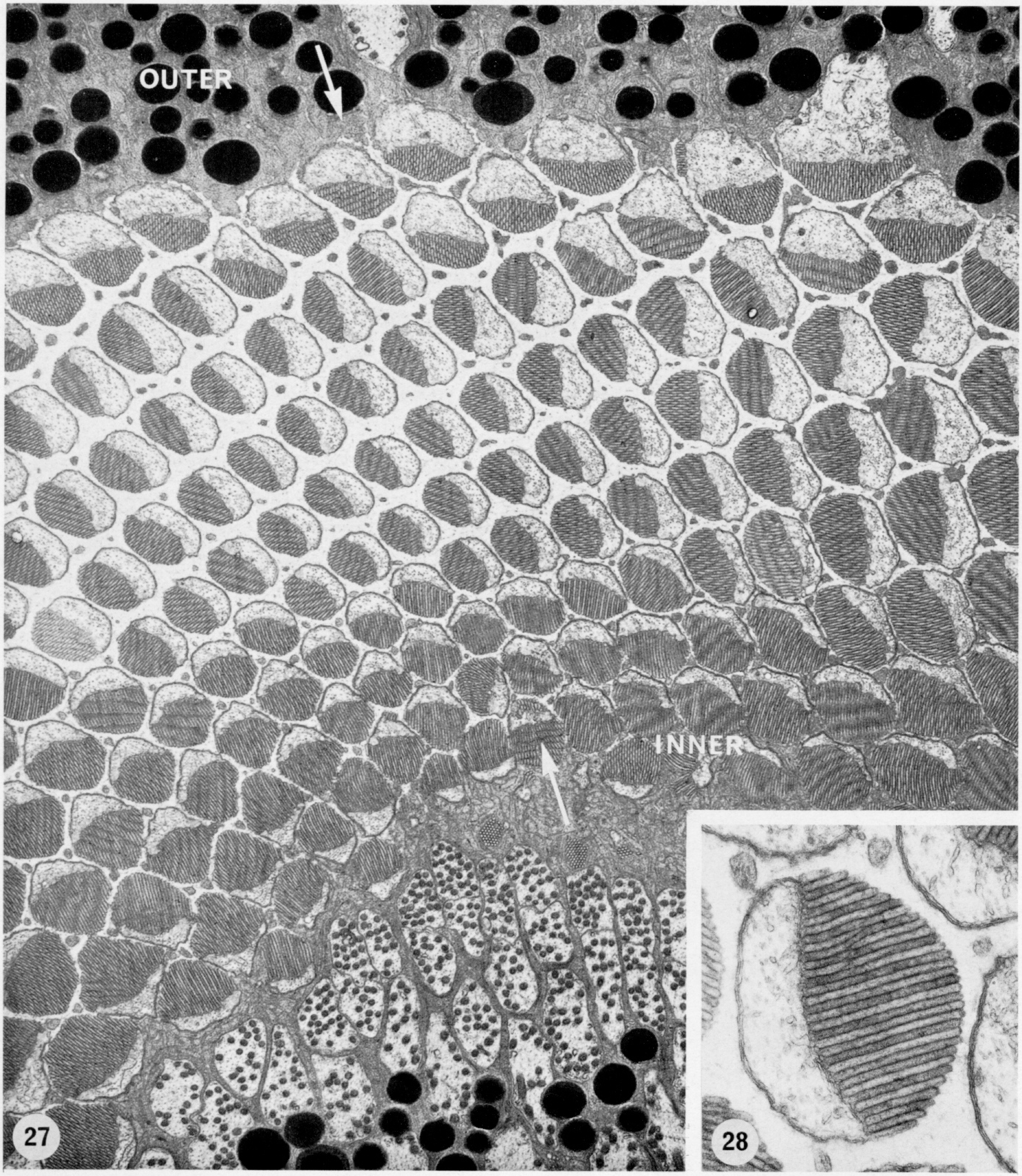

FIGURE 27. Low-magnification survey of Layer I receptive segments around the fovea, in transverse section, at day 15. (Magn. × 7500.)

FIGURE 28. Single Layer I receptive segment near to the fovea, in transverse section, at day 15. (Magn. × 25000.)

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FIGURE 29. Low-magnification survey of Layer II (2b) receptive segments around the fovea, in transverse section, at day 15. (Magn. × 8750.)

FIGURE 30. Layer II (2b) receptive segments with confluent rhabdomeres, in transverse section, at day 15. (Magn. × 25000.)