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

I. THE ESTABLISHMENT OF RECEPTOR TIERING BY CONFORMATIONAL CHANGES

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[Plates 1–6]

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The tiered principal retinae of salticid spiders are unique. No other arthropod ocellar retina resembles them anatomically, or sustains equivalently fine spatial acuities. The phylogeny of spiders guarantees that they evolved from far simpler, untiered retinae. The obscure systematic affiliations of the Salticidae preclude a search for extant forms that might offer precursors of the tiered retina, and it is probable that no such forms now exist. We examine the post-embryonic morphogenesis of a salticid principal retina, making a heuristic assumption that the concept of the ontogenetic recapitulation of phylogeny (Haeckel's 'Law of Recapitulation', now in a literal sense discredited) can, cautiously applied, either (1) indicate how the retina might have evolved, or (2) exclude some arbitrary models of its evolution.

By day 3 of post-embryonic development, a presumptive principal retina is established as a simple hemisphere of primordial receptive segments, to which new receptive segments are inferred to be still in the process of being added at the equator. At around days 4–5, the hemisphere of receptive segments starts rapidly to narrow. By around day 10, horizontal narrowing has transformed the initial hemisphere to an approximation of the mature, dorsoventrally aligned, boomerang-shaped retina. At day 12, spiderlings moult to the second instar, but conformational changes to the retina continue until day 15, when spiders leave their brood sacs and become independent.

The major conformational changes imply that distal receptive segments (e.g. those of Layers III and IV) are derived from those that initially lie at or close to the equator of the primordial hemisphere; and that foveal Layer I receptive segments remain at the retinal pole. Extreme horizontal narrowing ensures that equatorial receptive segments come to overlie those at the retinal pole, or at least near to it.

These findings exclude a hypothesis that receptors of different spectral sensitivities were randomly distributed over a primitive retinal hemisphere, and that tiering was generated during phylogeny simply by their differential migration along lines parallel to the optical axis. Nevertheless, limited local migrations of nascent receptive segments along such lines are inferred to contribute to the final pattern of tiering.

1. INTRODUCTION

The principal retinae of salticid spiders are supplied with images by a dioptric apparatus of long focal length, and lie at the ends of motile tubes (Land 1969*a*, *b*). A retina consists of a narrow, dorsoventral 'boomerang' with a small horizontal acceptance angle, and four tiers of receptors at the optical axis. Images received by the secondary eyes elicit turning responses by the spiders that bring objects of interest to the scrutiny of the principal eyes, whose limited fields of view are compensated by a variety of retinal movements (Land 1969*b*).

The successive hypotheses that have been advanced to explain how these eyes work will be outlined in the Discussion (§6). The purpose of this, and of the accompanying contribution (Blest & Carter 1988) is to describe in detail the post-embryonic morphogenesis of the principal eye of a phylogenetically advanced Australian jumping spider, *Plexippus validus*. Assuming some element of ontogenetic recapitulation, a clue to the phylogenetic origins of the Salticidae (currently, by any objective criteria, totally obscure) might be enshrined by the developmental history of their principal eyes. The context is as follows.

1. The tiered principal retina is unique both among spiders and the ocelli of arthropods in general. It can sustain extraordinarily fine spatial acuities that can amount to as little as 2.4′ (Land 1969*a*; Williams & McIntyre 1980; Blest 1985*a*). Ethological experiments indicate that such fine spatial acuities are necessary to account for the visual discriminations that the spiders are observed to make (Jackson & Blest 1982*a*).

2. The dioptric apparatus is a Galilean telescope, or telephoto lens system (Williams & McIntyre 1980; Blest *et al.* 1981).

3. Both tiering of the receptors and the unusual dioptrics must have evolved from the simpler principal eyes found in other families of spiders. Unfortunately, no bridging forms seem to be left to us. An examination of two species in the Spartaeinae and Lyssomaninae, subfamilies considered primitive by taxonomists (Wanless 1984) showed both to possess tiered retinae, although the architecture of Layer I, farthest from the dioptrics, is suboptimal (Blest & Sigmund 1984).

4. Haeckel's 'Law of Recapitulation' cannot be taken literally (De Beer 1958; Medawar & Medawar 1983), but there is a sense in which it can be conceptually useful. Selection pressures, if they are to modify an adult structure, must modify its morphogenesis. A developmental sequence may not exactly conserve the phylogenetic events that led to it, but can exclude some otherwise plausible hypotheses.

Homann (1971) described the embryonic development of the eyes of several families of spiders, and noted the formation of the retinal tube in the post-embryo of a salticid. He was not aware that the retina is tiered.

We show that the tiered principal retina is derived during post-embryonic development from a simple hemispherical arrangement of primordial receptive segments. They are reorganized to yield the narrow adult retina by conformational changes that imply that the distal receptive segments of the mature retina correspond to those that initially lie at the equator of the primordial hemisphere. A short preliminary account of this study and of the accompanying contribution (Blest & Carter 1988) has been published in Blest & Carter (1987).

2. MATERIALS AND METHODS

Brood-sacs of *Plexippus validus* (Urquhart) were collected from beneath eucalypt bark in Canberra, and held in the laboratory at 20 °C. Eggs were observed until the embryos shed their chorions. The resulting post-embryos were sampled from day 3 of post-embryonic development until day 15. Although post-embryos (which represent a first instar) moult to a second instar at day 12, we show that the retina continues to develop until day 15, when the spiderlings become independent and spontaneously leave their brood-sacs (Blest & Carter 1988).

All stages were dissected under a primary fixative solution which contained $25\ \mathrm{g\ l^{-1}}$ glutaraldehyde in 0.1 M cacodylate buffer with 0.09 M sucrose adjusted to pH 7.2 (Blest & Sigmund 1984). Primary fixation proceeded for 1–2 h at room temperature, after which tissues were post-fixed in OsO_4 ($10\ \mathrm{g\ l^{-1}}$) in the same buffer for 30 min, washed in distilled water, dehydrated through an ethanol series and propylene oxide, and embedded in Araldite.

The extent of dissection depended upon the age of the post-embryos. Initially, they are soft and readily deformed, so that it is only practicable to bisect their prosomae and remove the legs. Later, bisection of the prosomae could be followed by ablation of the chelicerae, and careful trimming of the anterior prosomal fragment so as to allow optimal penetration of the primary fixative.

For the gross analysis of retinal development, serial resin sections, 0.5–0.75 μm in thickness, were dried on slides in groups of five, stained with toluidine blue, and photographed with a Zeiss Photoscope equipped with a rotating stage, using Kodak Pan-X film developed in Kodak Microdol-X. A blue-green filter built into the sub-stage of the microscope was combined with an orange filter (Nikon, number 056) placed over the light source. To a human observer, the resulting illumination appears grass-green. It maximizes the contrast obtained on panchromatic black-and-white film for materials stained with toluidine blue.

Serial sections were cut frontally, so as to provide transverse sections of the developing retinal mosaics, or sagittally. Sagittal sections of a prosoma can be difficult to interpret: (1) in mature spiders, a retina is rotated around its optical axis by a special musculature (Land 1969*b*). During development the position of a retina with respect to that rotation is not precisely determined, and there are no landmarks that can indicate the position of a retina as it is sectioned; (2) similarly, principal retinae perform scanning movements in mature spiders (Land 1969*b*) and again, a developing retina is not predictably aligned with respect to its corneal lens. During the later stages of development, it is impossible to be sure that a given sagittal section is truly longitudinal with respect either to its component receptive segments, or to the future optical axis of the eye. Specific problems of interpretation are noted in §3.3. Here, it must be emphasized that serial sections at the level of resolution afforded by light microscopy do not allow unequivocal reconstructions of the early stages of the development of tiering. The preparation of strictly serial sections of retinae for transmission electron microscopy is clearly impracticable, given the number of stages that would need to be examined.

3. Background

3.1. *Gross anatomy of the mature retina*

A retina lies at the proximal end of a long tube derived from the hypodermis. The tube is filled with 'glass cells' which are responsible for the generation of the corneal lens (Eakin & Brandenburger 1971).

Between moults, the contents of a tube can be treated optically as composed of fluid with a refractive index of 1.336, that of physiological saline (Land 1969*a*; Williams & McIntyre 1980; Blest *et al.* 1981). The tiered receptive segments are embedded in a glial matrix of higher refractive index ($n \approx 1.4$) composed of the finely divided processes of cells whose somata lie proximal to the retina. In transverse sections, the matrix and the receptive segments present boomerang-shaped profiles at all levels, the arms of a 'boomerang' being directed dorsally and ventrally.

Land (1969*a*) designated the four tiers of receptive segments as Layers 1–4, Layer 1 lying farthest from the dioptrics. Blest *et al.* (1981) were concerned with co-tiering of receptive segments belonging to the major Layers, at the fovea, and also felt it desirable to distinguish between receptive segments whose somata lie at the outer and inner sides of the retina. They designated the major Layers as I–IV, but described each of their sub-fields as 'a' receptive segments (derived from somata at the outer side of a retina), and 'b' receptive segments (derived from somata on the inner side). Thus, for example, Layer II is composed of 2a and 2b receptive fields, each with a characteristic mosaic organization. Here we additionally designate peripheral Layer II$^{\bullet}$ receptive segments lying in the arms of a boomerang as '2c' for convenience. Similarly, the vertical strip of Layer IV (4a) receptive segments extends into the dorsal and ventral arms of the boomerang. Peripherally, such receptive segments are ultrastructurally distinct from 4a segments close to the fovea, and we designate them as '4c'.

The basic terminologies are given in table 1. Figure 1 (*a–e*) shows a schematic representation of the organization of a principal retina, with a diagram (figure 1*f*) that specifies the optical

TABLE 1. TERMINOLOGIES FOR THE FOUR MAJOR TIERS OF RECEPTIVE SEGMENTS IN THE PRINCIPAL RETINAE OF SALTICID SPIDERS

	Land (1969*a*)		Blest *et al.* (1981) receptive segment fields	
	tier	tier	central	peripheral
proximal	1	I	1	1
	2	II	2a, 3a	2c†
	3	III	3b, 4a	—
distal	4	IV	4a, 4b	4c‡

† As noted in the text, peripheral Layer II receptive segments are designated as '2c', for convenience.

‡ The peripheral extension of the dorsoventral strip of Layer IV (4a) is designated as '4c'. In these cells, the microvilli lie at 90° to those of 4a receptive segments (unpublished observations).

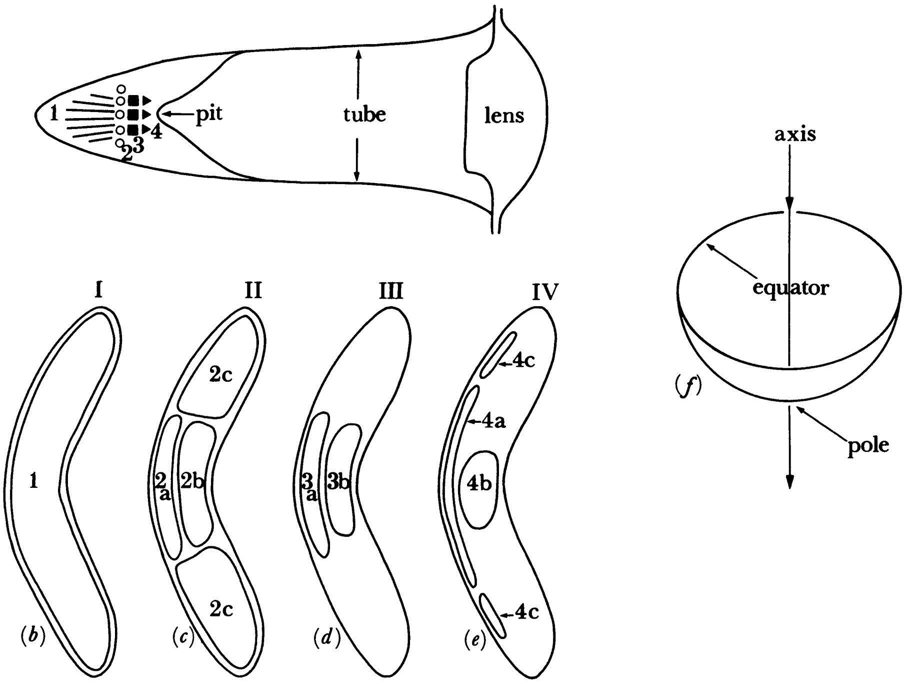

FIGURE 1. (*a*) Schematic longitudinal profile of a principal eye of an adult *Plexippus*. The retina lies remote from a corneal lens, at the proximal end of a retinal tube. It comprises four tiers of receptive segments at the fovea (1–4) that belong to the four major Layers of receptive segments (I–IV). A pit is formed by the interface between the glial matrix in which the receptive segments are embedded, and the contents of the retinal tube; it provides the diverging component of the telephoto dioptrics. (*b–e*) The fields of receptive segments belonging to Layers I–IV, schematized as transverse profiles at successive planes of section. (*f*) Definition of the terms used to describe a primordial retinal hemisphere.

axis, the equator and the pole of a retinal hemisphere. The matter of the precise pattern of tiering of 'a' and 'b' receptive segments belonging to Layers II–IV is irrelevant to this paper, although eventually it may prove to be optically important.

3.2. *Relevant optics*

Williams & McIntyre (1980) showed that the principal eye of a primitive spartaeine, *Portia*, is designed as a miniature Galilean telescope whose diverging component is provided by the interface between the glial matrix in which the receptive segments are embedded, and the contents of the retinal tube, which have been treated for the purposes of optical modelling as a fluid with a refractive index of 1.336 (that of physiological saline). The interface provides a small, proximal 'pit' whose apex is hemispherical. An apex overlies the strip of 4a receptive segments, those of the 3a and 2a fields, and the outer region of Layer I.

Blest (1985*a*) found similar telephoto optics in the principal eyes of some neotropical salticids inhabiting rain forest, and Blest *et al.* (1981) discovered the same optical arrangement in adult *Plexippus validus*. A re-examination of *Phidippus johnsoni* (one of the two species studied by Land (1969*a*,*b*)) has also revealed a pit with significant optical power (Blest *et al.* 1988). Thus the Galilean telescope is a general model for the construction of salticid principal eyes, and not a special case.

3.3. *Some technical problems of histological evaluation*

Traditionally, embryological studies of the present kind are done by making serial sections for light microscopy, and reconstructing the three-dimensional tissues that they sample. In the present context, there is a problem of resolution: even in semi-thin resin sections stained with toluidine blue, early receptive segments are small, and the boundaries between them poorly resolved. Orientation of principal eyes at all stages of development is also uncertain: (*a*) the precise position of a principal retina as it develops is loosely determined, and its alignment in relation to a rudimentary corneal lens cannot be determined from inspection of an embedded prosoma; (*b*) even for adult or sub-adult spiders, whose principal retinae can be dissected and handled individually, a retinal tube offers no external landmarks that can reliably predict how it should be oriented for sectioning.

Also, until day 10′, receptive segments are not only contiguous within the mosaics of a Layer, but at the fovea, receptive segments of adjacent Layers are longitudinally contiguous as well. The final axial spacing between Layers will be shown to be generated by an ingrowth of glial processes at a relatively late stage. Earlier, the close juxtaposition of receptive segments and the fuzzy boundaries between them as seen under the light microscope make it difficult to decide whether, to take an extreme example, four tiers of receptive segments are being viewed in a particular section, or whether a plane of section oblique to the future optical axis is sampling receptive segments from one Layer only, so giving an artefactual appearance of tiering. The problem resembles that posed by studies of the chemotaxis of the *Dictyostelium* grex (Odell & Bonner 1986): cells migrate with respect to each other, but cannot be individually recognized. Evidence for their patterns of movement can only be indirect.

4. RESULTS

4.1. *Criteria for stages of development*

Members of a given brood do not develop quite synchronously. Table 2 gives criteria used to define stages of development. They are less precise than one would wish. The appearance of the state of pigmentation of the secondary eyes as viewed under direct illumination gives approximations of the stages reached, but its interpretation is subjective. The appearance changes rapidly when spiderlings are preserved in ethanol (70% by volume), so that the progress of a given batch cannot be compared with stable standards.

We recognize some stages that can be reliably established from external criteria, and specify them, as, for example, day 7′. The latter notation indicates that days have been counted from the shedding of a chorion, but that variable rates of development do not allow an assumption that a given day presents spiderlings all at the same stage. More exact staging could only be

TABLE 2. CRITERIA FOR EVALUATING THE POST-EMBRYONIC DEVELOPMENT OF *PLEXIPPUS*

(Eclosion from the chorion takes place at day 0. Subsequent stages are assigned in terms of the acquisition of pigmentation by the anterior lateral and posterior lateral eyes, the development of hypodermal pigmentation, and development of the second instar tarsal claws, whose state can be observed from whole mounts of legs sampled from days 8′–11′, by transmitted light.

Acquisition of pigment granules by the pigmented glia of a principal eye does not lend itself to diagrammatic representation. It is indicated as: +, faint pigmentation, to + + + + +, strong, final pigmentation. For developing anterior lateral and posterior lateral eyes, pigmentation is schematized as seen in prosomae immersed in primary fixative.)

day	anterior median eye	anterior lateral eye	posterior lateral eye	hypodermis, etc.
0	—	—	—	—
1–2	+	—	—	—
3	+ +	—	—	—
4′	+ + +	glial pigment appears in both eyes as very faint crescents		a very faint pattern appears
5′	+ + + +	○	☾	
6′	+ + + +	⭘	○	the hypodermal pigmentation starts to consolidate
7′	+ + + +	◉	⭘	
8′	+ + + + +	◉	◉	
9′	+ + + + +	●	●	2nd instar tarsal claws are just visible
10′	+ + + + +	●	●	2nd instar tarsal claws are fully formed
11′–12	+ + + + +	●	●	2nd instar tarsal claws are fully sclerotized

moult to second instar

achieved by examination of the ultrastructure of large series of retinae by electron microscopy, a task that is obviously impracticable.

The temporal imprecision does not matter, because we are primarily concerned with establishing a sequence of events. Pragmatically, we are safe when designating stages up to day 3 thus, because relatively few significant events occur during the first three days of post-embryonic development. The moult to the second instar occurs at day 12, and days 13–15 can be reliably specified from that point. Days 4′–11′ must be taken as imprecisely defined. Our solution is a compromise between what is observed to happen and a sequence of events that can be reliably inferred to take place.

4.2. *Gross post-embryonic development of the principal retina*

Until day 3, serial, semi-thin sections show a primordial retina to consist of an aggregate of undifferentiated cells which Homann (1971) showed to derive from an invagination of the blastoderm. At no post-embryonic stage are either mitotic figures or degenerating cells observed. Morphogenesis of a principal retina must, therefore, derive solely from the differentiation of a population of primordial cells and from the interactions between them.

Day 3

A primordial principal retina consists of a hemisphere of nascent receptive segments disposed as a monolayer. They present a characteristic 'honeycomb' appearance in transverse section (figures 2–7, plate 1). The receptive segments are flanked by strands of material that appear punctate in light micrographs (figures 2–6). Electron microscopy reveals them as outgrowths from the somata of the future pigmented glia (Blest & Carter 1988). The dendrites that supply nascent receptive segments from neuronal somata proximal to the retinal hemisphere cannot be distinguished in light micrographs. The future optic nerve leaves the retina dorsally, and is labelled in figure 2.

Figures 2–7 display a retina early in day 3. Nascent receptive segments are fairly evenly distributed around the hemisphere. Figures 8–13, plate 1, offer sections from a retina sampled notionally late in day 3. There is a concentration of nascent receptive segments ventrally, anticipating later morphogenetic events, all of which are initiated at the ventral region of the hemisphere.

Days 4′–5′

The primordial retina undergoes a dramatic conformational change (figures 14–21, plate 2). It narrows in a manner that anticipates the final conformation of the mature retina, starting ventrally. Thus at the outer and inner sides of the retina, primordial receptive segments move inwards along a horizontal line so that eventually they will come to overlie the retinal pole.

The eye possesses a rudimentary corneal lens (figure 20) that cannot, because of its shape, be optically effective. The proximal region of the retinal tube contains numerous nuclei of the glass cells, lying in the future optical pathway (figure 14). There are, however, no large dense bodies in the glass cells; they appear later.

Day 6′

The sequence of sagittal sections shown by figures 22–28, plate 3, reveals that a significant degree of tiering has been established. Although it is reasonable to assume that the long receptive segments towards the left of each micrograph represent future foveal or near foveal Layer I receptive segments, they cannot at this stage be reliably attributed to Layers.

The series of transverse sections in figures 29–33, plate 3, show that differentiation is most advanced at the outer side of the retina. Figures 25–27 indicate that the glass cells now contain distinguishable dense bodies.

Day 7′

The consolidation of tiering is well-advanced, but is not interpretable from light micrographs. The glial matrix remains poorly developed, so that distal receptive segments at the fovea are still closely apposed to the proximal end of the retinal tube. Interpretation of the final adjustments that lead to the mature pattern of tiering rests upon electron micrographs of

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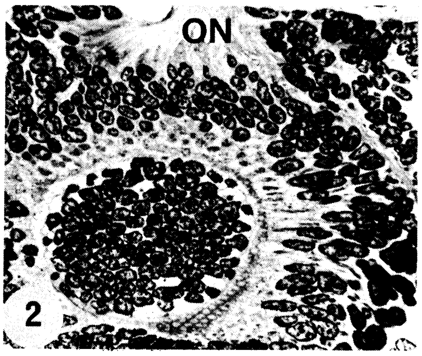
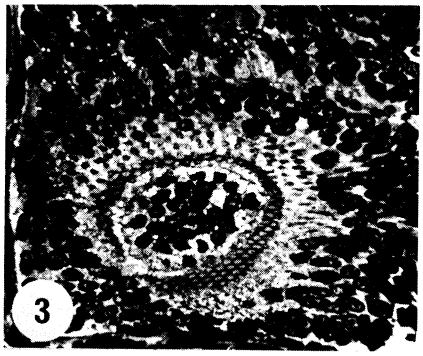
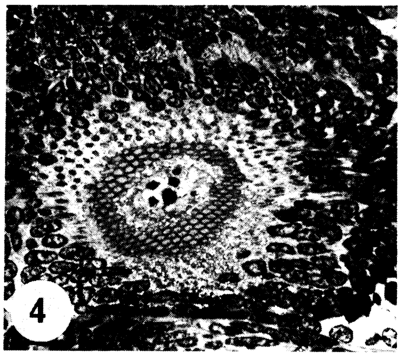
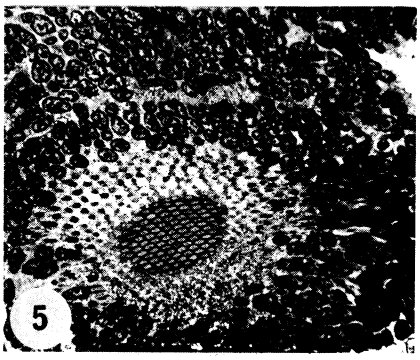
DESCRIPTION OF PLATE 1

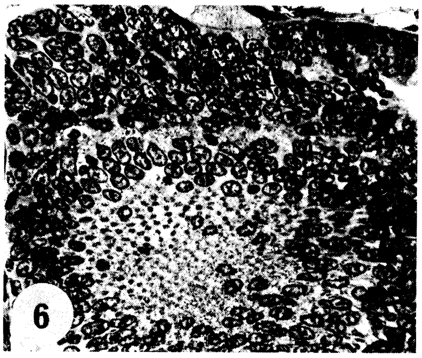
FIGURES 2–6 and 8–12 represent sections of principal retinae at day 3. Figures 2–6 derive notionally from a retina early in day 3, whereas figures 8–12 represent a slightly later stage of development. Figures 7 and 13 provide scales and orientations for the two series of sections.

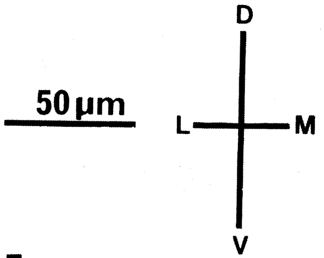












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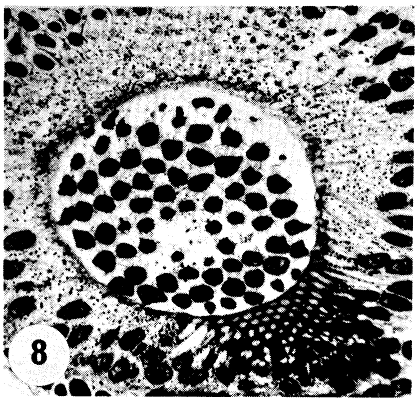

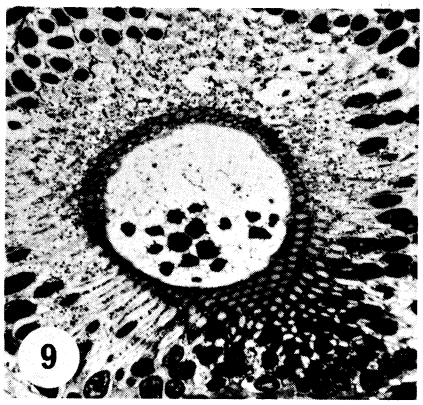

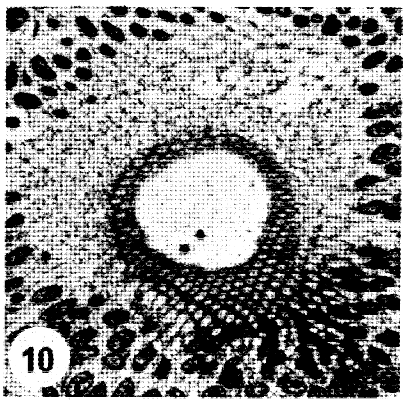

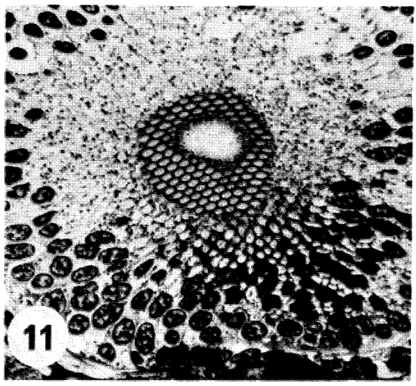

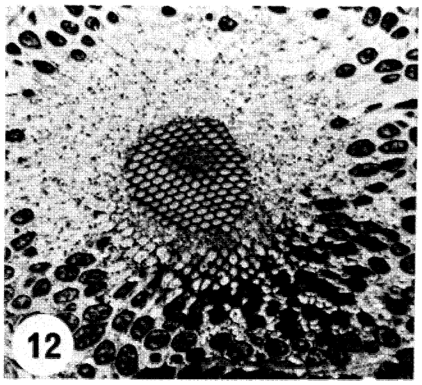

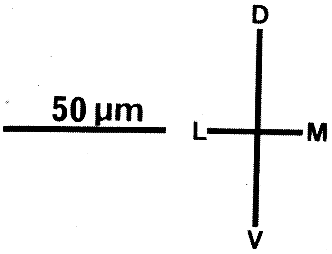

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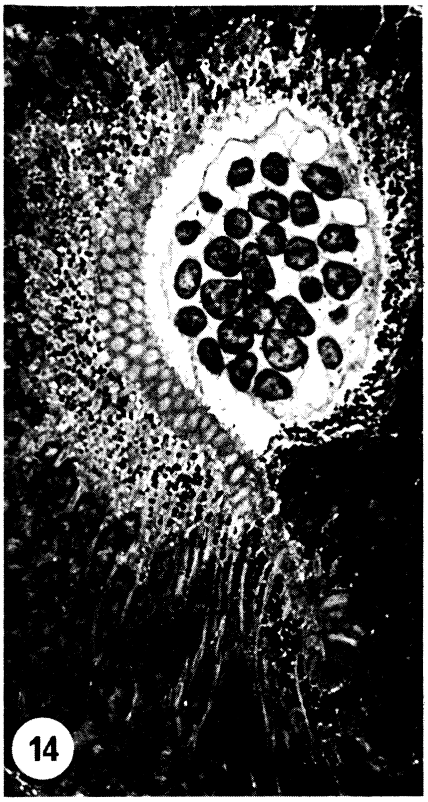
FIGURES 2–13. For description see opposite.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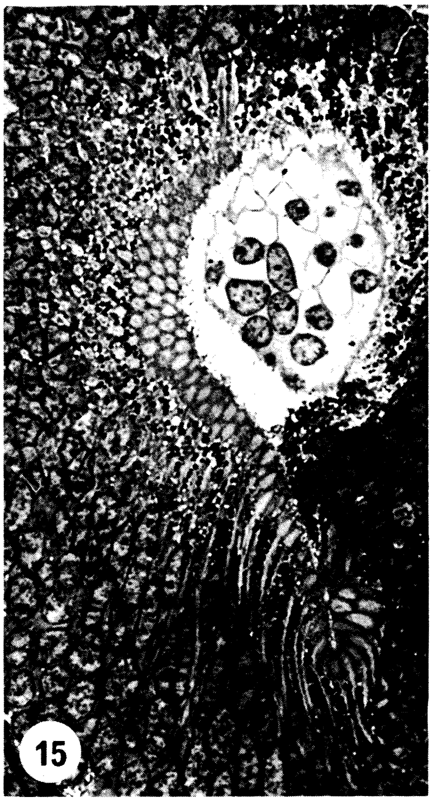
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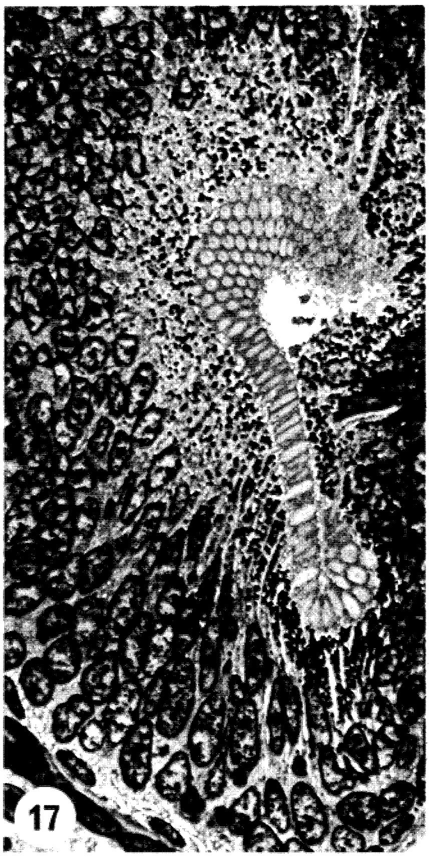
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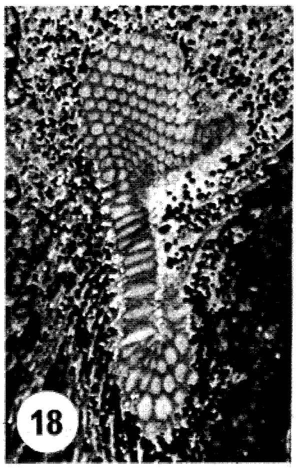
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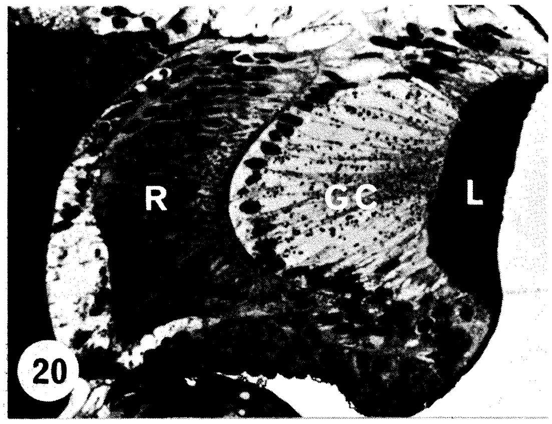

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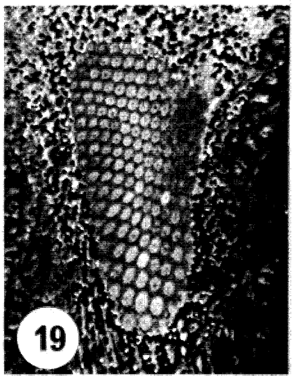
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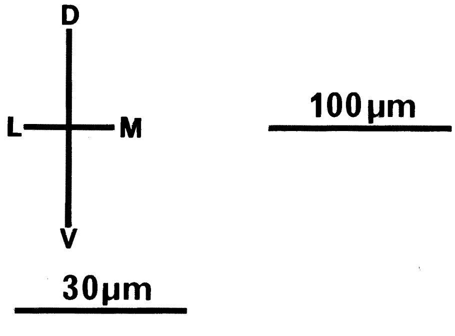

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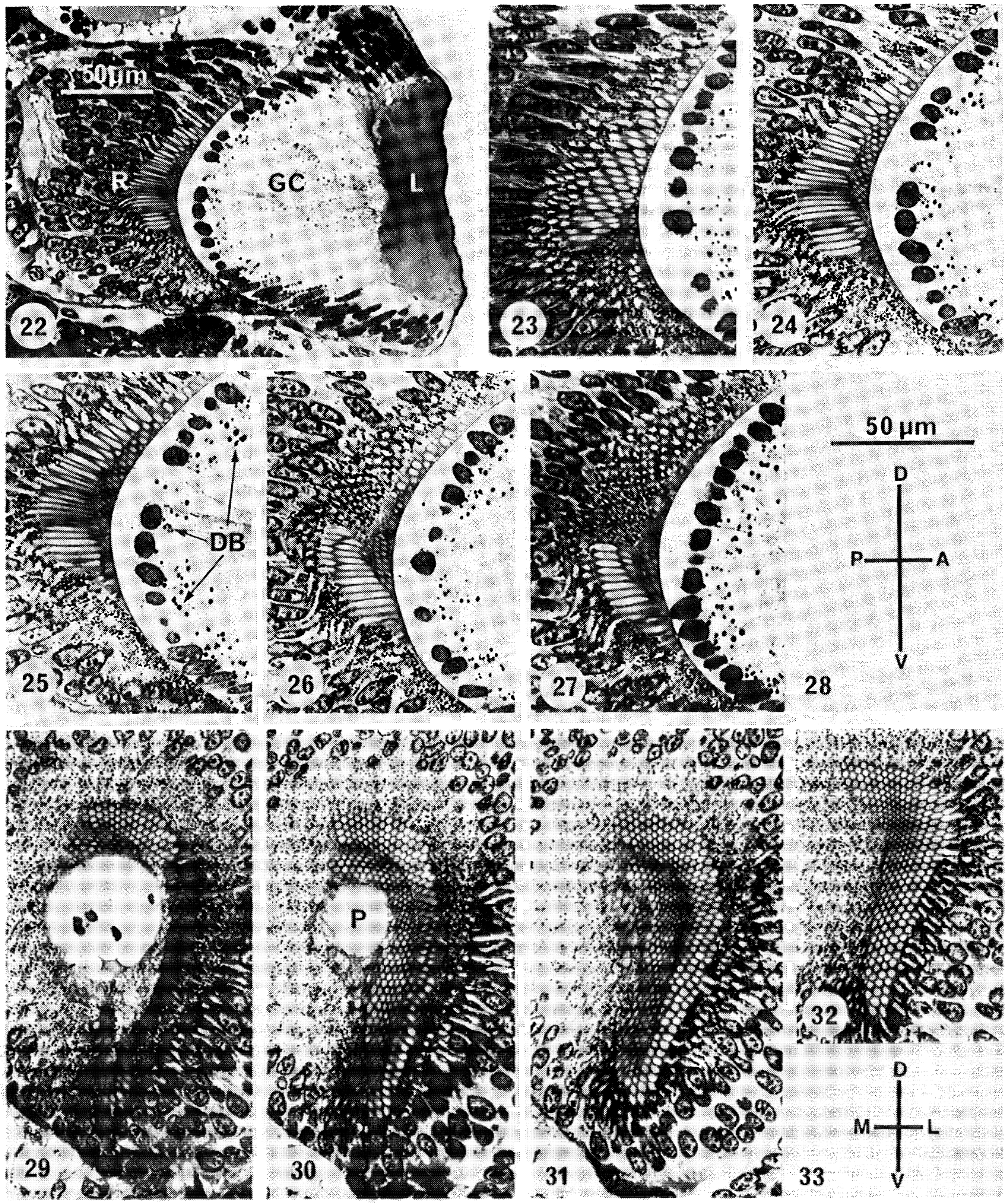
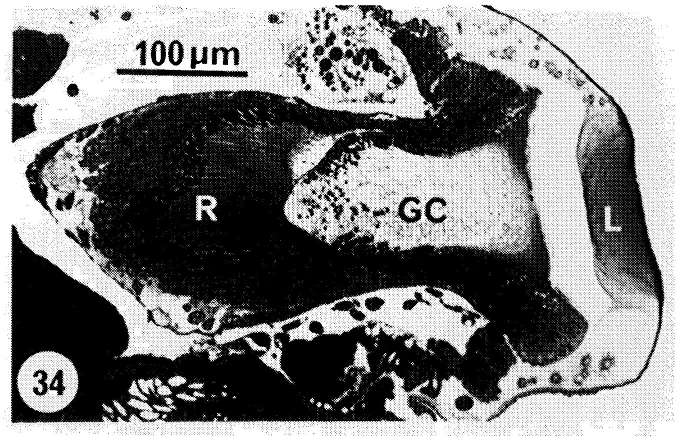
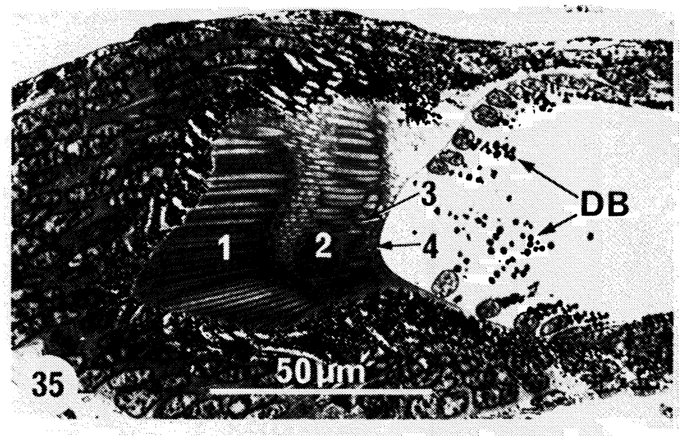
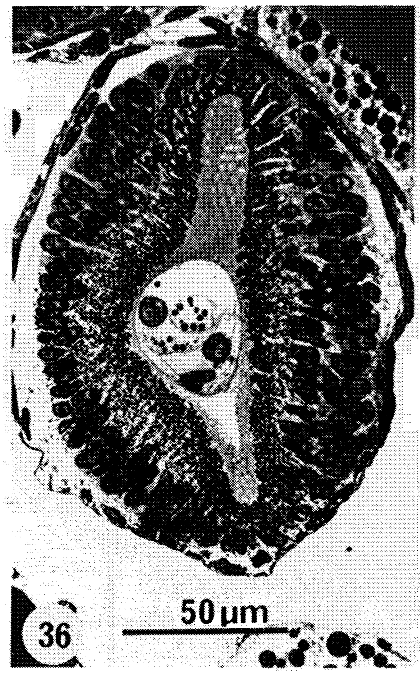
FIGURES 14–21. For description see facing plate 4.

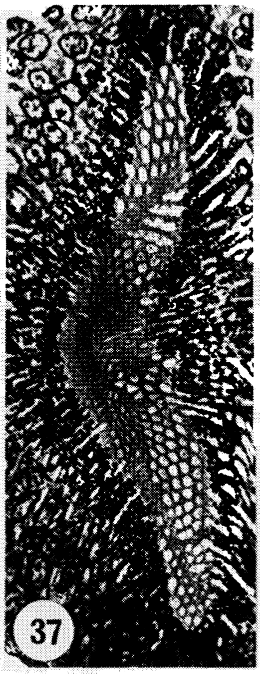


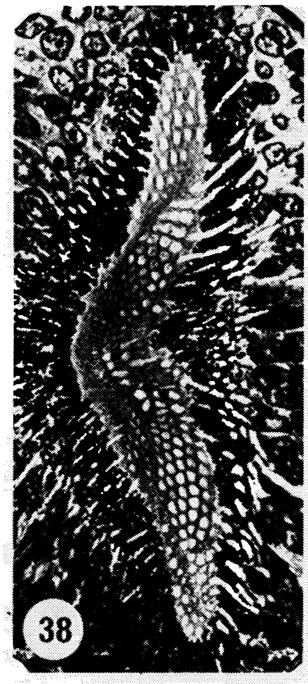
FIGURES 22–33. For description see facing plate 4.

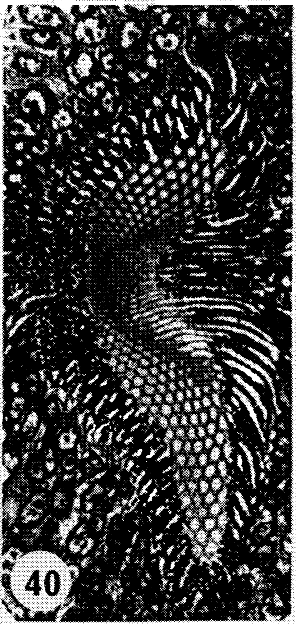


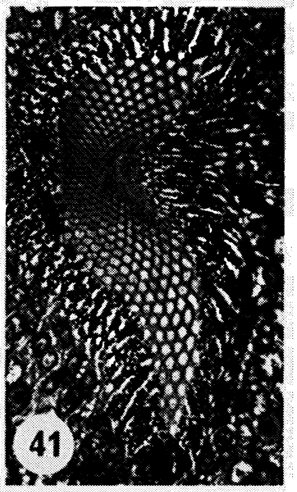


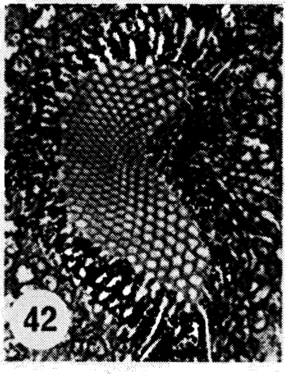


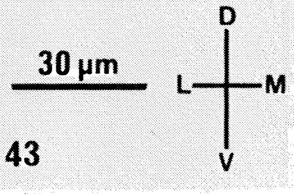












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FIGURES 34–43. For description see opposite.

DESCRIPTION OF PLATE 2

FIGURES 14–19. Transverse sections of a principal retina at day 4′–5′, at the inception of tiering.

FIGURE 20. Sagittal section of an entire principal eye at the same stage.

FIGURE 21. Scale and orientation for figures 14–19 (left) and a scale for figures 20 (right). Full explanation in the text.

DESCRIPTION OF PLATE 3

FIGURES 22–27. Sequence of sagittal sections through a principal retina at day 6′, to illustrate the early pattern of tiering. Receptive segments are not attributed to Layers, because at this stage they cannot be reliably determined.

FIGURE 28. The orientation of figures 22–27, and a scale for all figures of plate 3.

FIGURES 29–32. Sequence of transverse sections of a principal retina at day 6′.

FIGURE 33. The orientation of figures 29–32. Full explanation in the text.

DESCRIPTION OF PLATE 4

FIGURES 34–43. Sections of principal retinae at day 11′, just before the moult to the second instar.

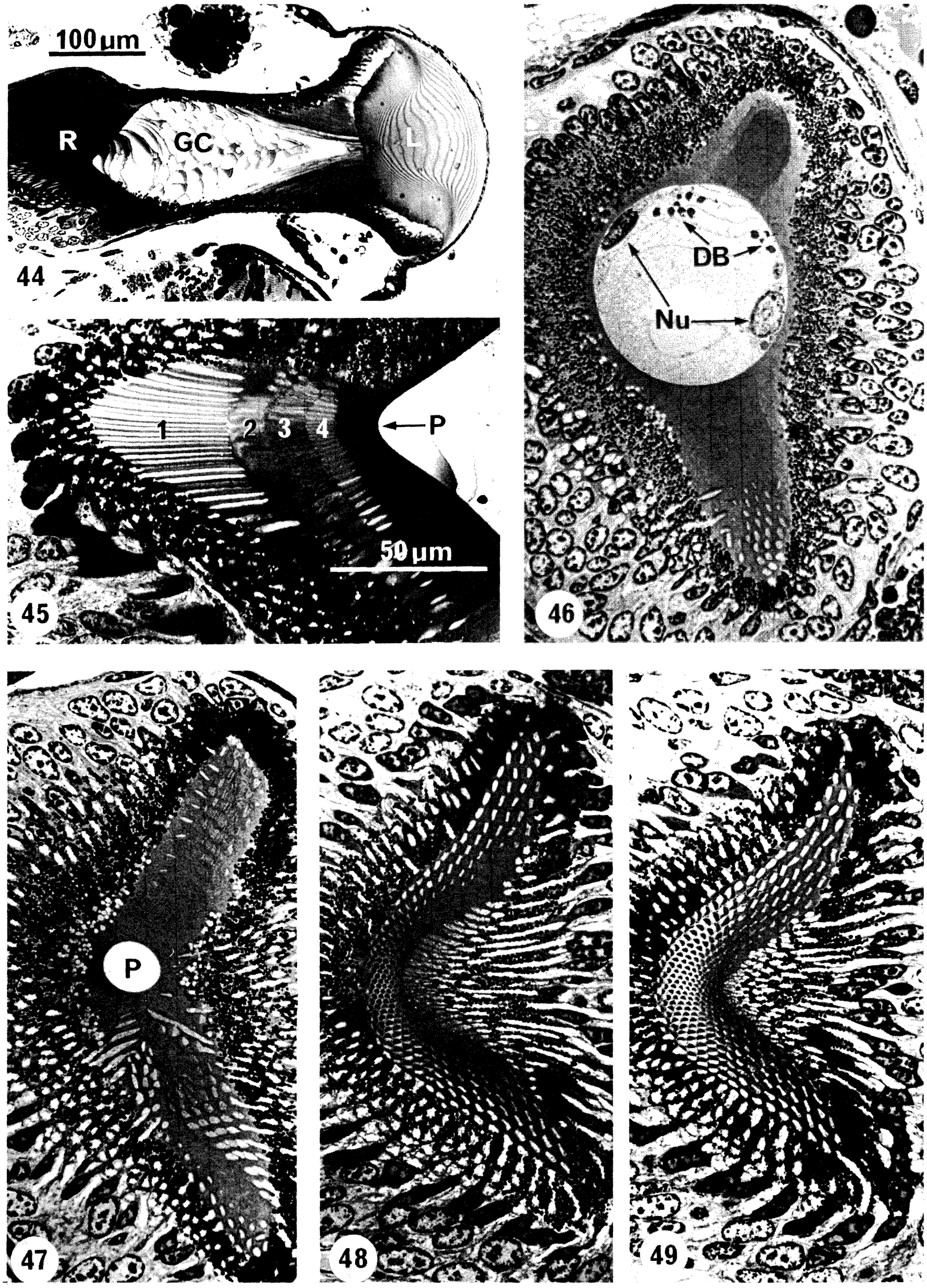
FIGURE 34. Near-axial sagittal section of an entire retina.

FIGURE 35. Layers I and II are well-established, but Layers III and IV are still shallow and incompletely differentiated.

FIGURE 36. Distal transverse section of a principal retina; glass cells are still occupied by nuclei and dense bodies close to the 'pit' and the optical axis.

FIGURE 38–42. Sequence of transverse sections through a principal retina; the final 'boomerang'-shaped conformation has not been achieved, but tiers of receptive segments are in place.

FIGURE 43. Scale and orientation for figures 37–42. Full explanation in the text.



FIGURES 44–49. For description see opposite.

DESCRIPTION OF PLATE 5

FIGURES 44–49. Sections of principal retinae at day 15.

FIGURE 44. Sagittal section of an entire eye.

FIGURE 45. Tiering of the receptive segments and the organization of their glial matrix is complete.

FIGURES 46–49. Sequence of transverse sections of a principal retina.

FIGURE 46. Nuclei and dense bodies lie at the periphery of the retinal tube.

FIGURE 47. Optically critical region of the 'pit' has been cleared of organelles.

FIGURES 48 AND 49. Mature, transverse, 'boomerang'-shaped profile of the principal retina has been achieved. Full explanation in the text.

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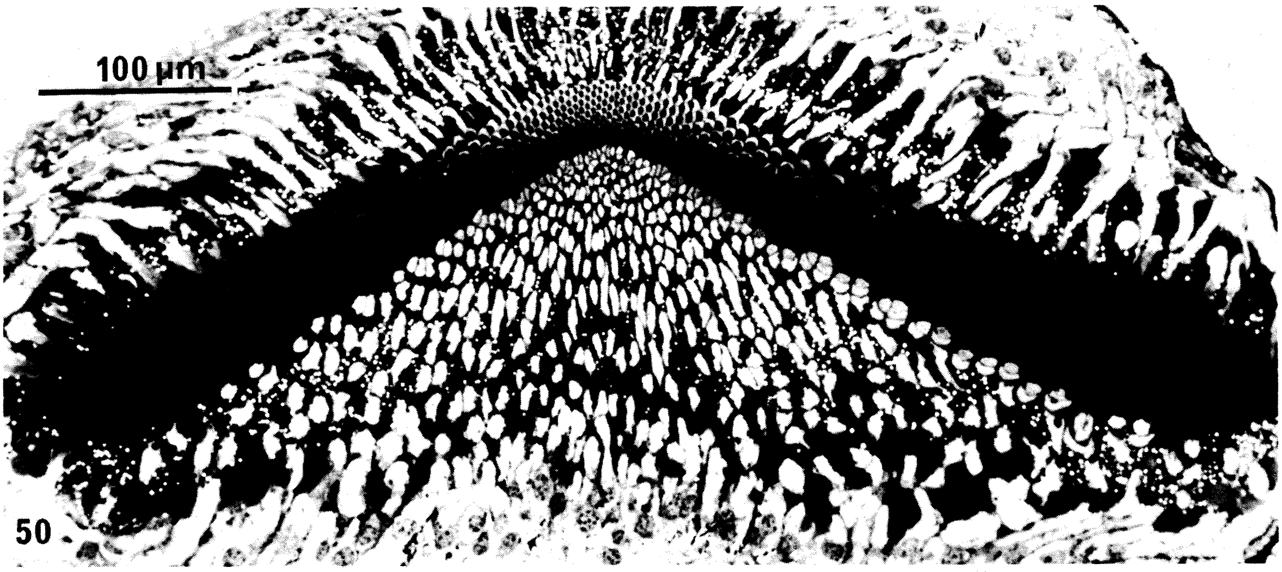

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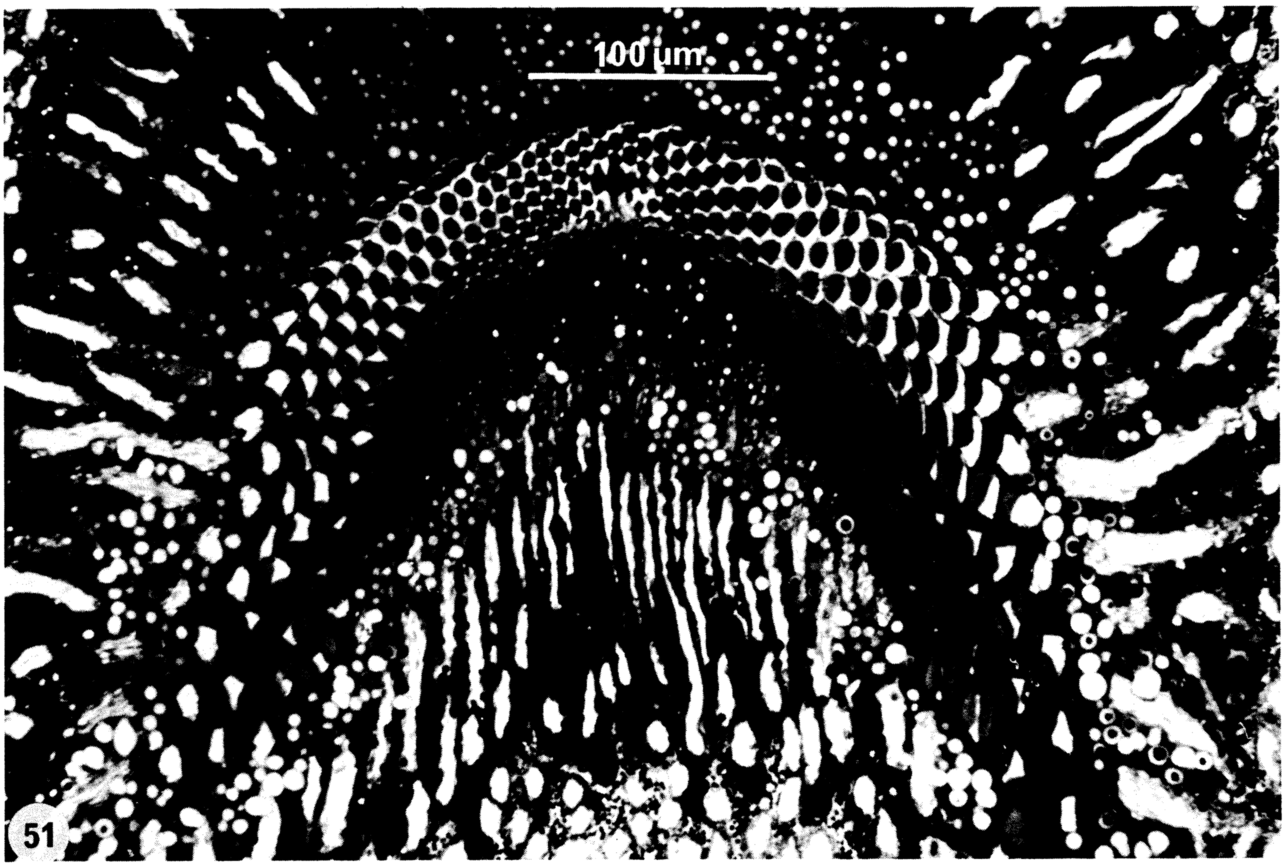

FIGURES 50 and 51. For description see opposite.

transverse sections taken at days 7′ and 10′. An accompanying paper (Blest & Carter 1988) deals with the ultrastructure of the developing retina, and displays low-magnification electron micrographs of transverse sections of the four Layers of receptive segments at the fovea, at successive stages of development. At day 7′, immediately adjacent to the retinal tube, primordial 4a, 3a, 3b and 4b receptive segments lie at the same plane along a horizontal line that traverses the fovea (Blest & Carter 1988, figure 3).

Day 10′

Blest & Carter (1988, figure 13) illustrate a transverse section taken at the same plane as the section discussed above. Along a horizontal line that traverses the fovea, 4a receptive segments still lie adjacent to those of 3b, but the latter are now flanked on the medial side of the retina by 4b receptive segments.

Day 11′

Figures 34–43, plate 4. Day 11′ immediately anticipates the moult to the second instar at day 12. Figure 34 shows that the lens is separated from the contents of the retinal tube by a space occupied by moulting fluid. Figure 35 represents a sagittal section of the retina, taken close to the optical axis. Layers I and II are well-established and readily distinguishable. Layers III and IV are barely differentiated, and are still closely apposed to the apex of the pit.

Figure 36 shows that quite near to the apex of the pit, the future light path is still occluded by nuclei and dense bodies. Figures 37–42 present transverse sections of a retina, starting at distal planes (figures 37–39) that include developing Layer III and IV receptive segments at the fovea. The transverse conformation is a poor approximation of the 'boomerang' achieved at day 15 (figures 44–49, plate 5).

Day 15

At day 15, spiderlings leave their brood-sacs and become independent. Figure 44 shows that there is a fully developed lens and a long retinal tube. Figure 45 presents a near-axial sagittal section of a retina. The four tiers of receptive segments are in place, and Layer IV (4a) receptive segments are separated from the apex of the pit by the glial matrix. Glass cells lying in the optical pathway are axially devoid of organelles. Figure 46 shows that nuclei and large dense bodies lie peripherally at the margins of the retinal tube, and figure 47 shows that the pit is also devoid of organelles. Figures 48 and 49 represent transverse sections of Layer I, at the distal tips of the rhabdomeres (figure 48) and near to the mid-point of Layer I (figure 49).

5. INTERPRETATION

That tiering of the receptive segments is primarily generated by their migration along a horizontal line towards the future optical axis can be inferred from the narrowing of a retina

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DESCRIPTION OF PLATE 6

Two transverse configurations of principal retinae are compared.

FIGURE 50. Transverse section of a retina of *Phidippus otiosus* taken at the level of distal Layer I.

FIGURE 51. Equivalent section of a retina of *Ocrisiona* sp. Full explanation in the text.

from a distal diameter of *ca.* 90 μm at day 3 (e.g. figure 8) to a horizontal width at the fovea of some 20 μm at day 11′, just before the moult (figures 37–40). Because primordial cells are not deleted during post-embryonic development, it must be assumed that the ultimate conformation of a principal retina results from interactions between cells destined to provide the pigmented glia and the glial matrix of the retina, and those that differentiate to provide the four Layers of receptive segments. Thus the primordial glial and receptive cells must collaborate to make the developing retina narrow, presumably as a consequence of mechanical forces exerted upon the initial retinal hemisphere. It is economical to suggest that growth of the glia provides them. A schematic outline of the gross conformational changes between days 3 and 11′ is given in figure 52.

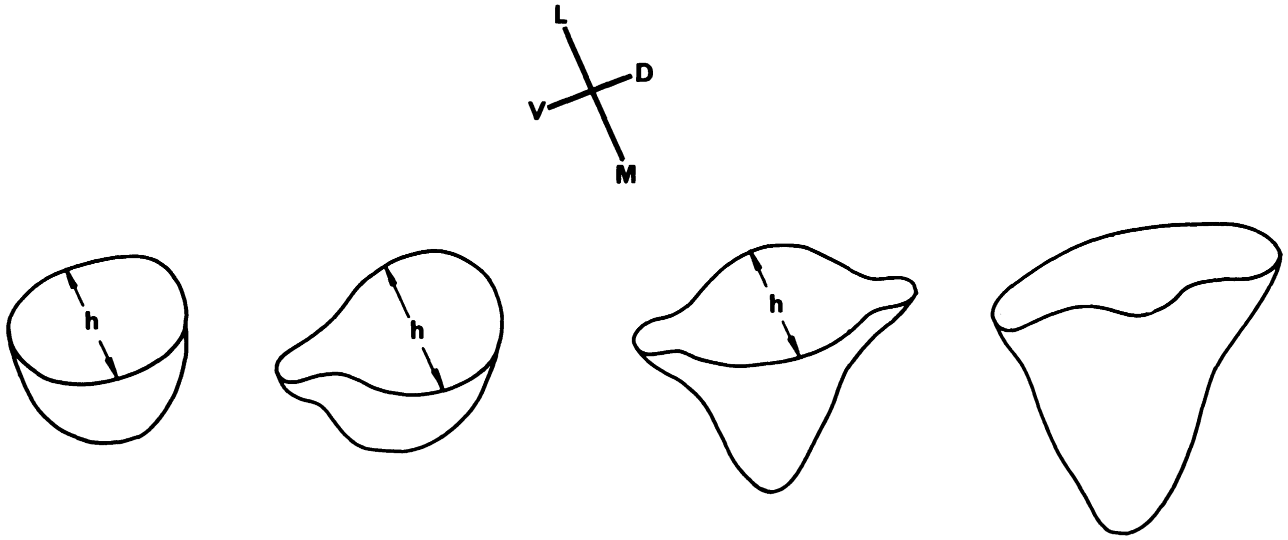

FIGURE 52. The gross conformational changes that a developing principal retina undergoes between day 3 and day 11 of post-embryonic development are schematized, reading from left to right. The orientation of the diagrams is given at the top of the figure: L, lateral; M, medial; V, ventral; D, dorsal. 'h' indicates a horizontal line across the mid-point of a retina.

Whatever determines narrowing, the gross configurational change accounts quite satisfactorily for the final disposition of receptive segments in the four Layers. If receptive segments of Layers III and IV are derived from receptors that originally lie at local regions on the equator of a hemisphere, horizontal narrowing would account for their concentration at the fovea, for simple topological reasons. Some possible phylogenetic parallels are explored in detail in §6.3.

Horizontal narrowing of a retina does not adequately explain the final tiering of receptive segments. As has been noted above (§4.2) there must be a limited migration of nascent receptive segments along lines parallel to the optical axis.

6. DISCUSSION

6.1. *Phylogenetic background*

The Introduction noted that the phylogenetic origins of the Salticidae are unknown. Objectively, the evolutionary pathway that led to the evolution of the unique principal eye has remained unresolved. An obvious speculation about the origins of the Salticidae might suppose them to represent an evolutionary elaboration of diurnal, cursorial spiders that initially possessed simple, untiered principal retinae. Such a hypothesis would assume that independence from webs and a cursorial habit preceded the phylogenetic fine-tailoring of the principal eyes that must have occurred. There is, however, little to recommend it. Existing cursorial spiders (for example the Lycosidae and Oxyopidae) have small principal eyes with a simple retinal architecture (Bacetti & Bedini 1964; Homann 1971; Blest 1985*b*). Their secondary eyes are quite well developed, command substantial fields of view and have fairly satisfactory receptor mosaics (Blest & Day 1978; Blest, 1985*b*; Land 1985).

An alternative hypothesis for the evolution of the Salticidae has been proposed by Jackson & Blest (1982*b*). The specialized ethology of a primitive spartaeine salticid, *Portia*, suggests that the Salticidae may have arisen directly from web-building spiders that acquired the tactic of invading webs belonging to other families of spiders. The authors argued that it would have been of selective advantage to supplement kinaesthetic cues to the position of an alien, resident spider with visual information. On that basis, because information conveyed by an invaded web might be expected to indicate the approximate direction but not distance of an occupant, the principal eyes, pointing ahead of a spider, would have been elaborated first, the evolution of the secondary eyes lagging behind.

Such evidence as we have supports the latter hypothesis. *Yaginumanis* and *Lyssomanes*, both regarded as relatively primitive by taxonomists (Wanless 1984) have tiered principal retinae whose efficiency is nevertheless compromised either by limited light-guide construction at the fovea (*Lyssomanes*) or by its total absence (*Yaginumanis*) (Blest & Sigmund 1984). Their secondary eyes are, indeed, less efficiently designed than those of the advanced species that have so far been studied (Eakin & Brandenburger 1971; Blest 1983, 1984).

6.2. *Functional background*

A major problem is: how does the tiered principal retina work? It is helpful to summarize the functional models that have been suggested to explain retinal tiering, in the order in which they were proposed.

1. *Retinal tiering compensates for lack of accommodation*

Land (1969*a*) suggested that if all but Layer IV receptive segments had peak responses to green light, retinal tiering could compensate for the inability of the eye to accommodate. Each Layer would receive focused images from a different plane in object space, such that distances from infinity to planes close to the spiders would be covered.

2. *Retinal tiering compensates for the chromatic aberration of the corneal lens*

As an alternative to (1), Land (1969*a*) suggested that for distant objects tiering could compensate for the measured chromatic aberration of the corneal lens. In that case, Layer I would have peak responses at long wavelengths in the red–orange.

Both models require Layer IV receptors to have peak responses in the UV.

The anatomical data upon which the two models were based have since proved to be incorrect (Blest *et al.* 1988). In particular, the light-guiding properties of foveal Layer I rhabdomeres were not appreciated, and the poor mosaic qualities of Layers II–IV were not understood.

3. *Layer I alone compensates for lack of accommodation, at the fovea*

Blest *et al.* (1981) remodelled a principal eye in terms of its telephoto optics and a new anatomical finding that the tips of Layer I rhabdomeres are longitudinally 'staircased' with respect to a horizontal line at the fovea. They also showed by dye-marked intracellular recordings that Layer I and II receptors have peak responses in the green, and Layer IV receptors in the UV. Recordings from Layer III were not obtained.

If the whole of Layer I consists of green receptors, and all foveal Layer I rhabdomeres are light guides, the retina can be modelled on an assumption that the 'staircase' of Layer I rhabdomeres allows them to receive focused images at their tips of objects from infinity to around 2 cm in front of a spider, the outer region of a retina handling images from distant targets and the inner region of those nearby. The model is justified, because it is clear that the poor mosaic qualities of Layers II–IV at the fovea are inadequate to sustain the fine visual discriminations that the spiders are observed to make (Jackson & Blest 1982*a*).

In this model, Layer IV still receives focused images in the UV, but it does not prove possible to infer a satisfactory optical role for Layer II.

6.3. *Alternative models for the morphogenesis and implied evolution of salticid principal retinae*

We assume that the tiered principal retina evolved from an untiered precursor, and that it is improbable that the morphogenetic route described in the Results differs radically from the evolutionary pathway that led to it. Ideally, an explanation of either would take into account the distribution of receptors of different spectral types in the principal eyes of other families of spiders. Little is known about them, except that Yamashita & Tateda (1978) demonstrated green, blue and UV receptors in the principal eye of an argyopid. Their result has not been confirmed for a congeneric species by Tiedemann *et al.* (1986) whose electroretinograms implied a population of green receptors only.

If we suppose that a precursor of the salticid principal retina possessed green, blue and UV receptors how it might have evolved would have depended upon their initial distribution in a retinal hemisphere. There are two plausible hypotheses.

1. *Ancestrally, spectral types were randomly distributed across a retina*

Tiering would be derived most readily by the differential migration of receptive segments along lines parallel to the optical axis, such that eventually, foveal green receptors either remained at the retinal pole (Layer I) or close to it (Layer II), and notionally blue receptors (Layer III) and UV receptors (Layer IV) adopted positions progressively further from the pole.

The model is attractive, because it supposes that different spectral types could have migrated in a simple way that would have followed the progressive increase in the focal lengths of the corneal lenses, and the evolution of the long retinal tube. The greater the focal length of a corneal lens, the more severe the consequence of its chromatic aberration for the retinal mosaics to which it delivers images.

The model is unattractive, firstly because it is not consistent with the morphogenetic pattern that we have described, and it does not explain the positions of intermediate segments in mature retinae. Those of Layer I enter the retina at its proximal end and lie more or less parallel to the optical axis. All others enter laterally, more or less at right angles to it.

Secondly, it does not explain the clear distinction in Layers II–IV between fields of receptive segments derived from somata on the outer side of a retina ('a' receptive segments) and those derived from somata on the inner side ('b' receptive segments).

2. *Ancestrally, different spectral types were each confined to a local retinal domain*

If UV-receptive segments were initially distributed around the equator of a retinal hemisphere, narrowing of a retina would cause them to overlie the retinal pole. Because we observe such a migration during morphogenesis, this is obviously the most attractive phylogenetic model, for it also accounts for the orientation of the intermediate segments of Layers II–IV. We do not know how UV-receptive segments are distributed in any simple spider ocellus; intracellular recordings made by Yamashita & Tateda (1978) were from unmarked cells.

A speculation that assumes that UV-receptive segments originally lay at the equator of a retinal hemisphere permits a discussion of an unexplained feature of the salticid principal retina. Despite the optically unsatisfactory foveal Layer I mosaics in *Lyssomanes* and *Yaginumanis* and the 'transitional' Layer I mosaic of *Spartaeus* (Blest & Sigmund 1984, 1985), all salticids that we have examined, 'primitive' or 'advanced', possess the same arrangement of Layer IV (4a) receptive segments: a dorsoventral strip with ordered microvilli that Eakin & Brandenburger (1971) noted as well suited to sustain polarization analysis.

1. Polarization analysis is only required for navigation that exploits the polarization pattern of the sky. Görner & Claas (1985) summarized experiments that showed an agelenid, *Agelena labyrinthica*, to use sky polarization when returning on its sheet-web to a retreat after an excursion to catch prey. Polarization analysis was shown to be done by the principal eyes. The same authors noted previous studies of Lycosidae and of the orthognathate *Aphonopelma* that indicated similar capacities to exploit polarization cues during navigation.
2. Schröer (1974, 1975, 1976) inferred, from anatomical data, that in *Agelena* polarized light is analysed by a sub-population of cells in the ventroperipheral region of a principal retina. Optically, that region would survey the sky. Functionally, it would correspond to the small field of UV receptors at the dorsal rim of the compound eye of honey-bees that survey the sky and conduct polarization analysis (Rossel & Wehner 1986).
3. Hill (1979) failed to find a role for polarization analysis in the prey-catching behaviour of a salticid, *Phidippus pulcherrimus*. Spiders used local, topographic cues when approaching prey across a complex, disrupted, three-dimensional terrain, and ignored experimental disturbances of the pattern of sky polarization while doing so. Despite Hill's ingenious experiments, the task may have been inappropriate, for it does not correspond to that required of *Agelena* when it returns across a web to a retreat the position of which is fixed.
4. Salticids reside in silken refuges at night, and must, presumably, navigate if they are to return to them. Little attention has been paid to the permanence of these refuges, or to the distances from them at which the spiders perform their diurnal activities. Observations of *Phiale magnifica* Banks in Panama (A. D. Blest, unpublished results) suggest the following situation. Firstly, sub-adult and adult refuges are so robustly constructed that certainly days, and more probably weeks, must be spent building them. An adult *Phiale magnifica* may be seen hunting for prey at substantial distances from its permanent refuge, from which it may be separated by a broken terrain whose instability (e.g. branches moved by wind) does not seem to favour return navigation either by landmarks or by dead-reckoning.

5. Thus it is not unreasonable to suggest that some salticids use the pattern of sky polarization to orient them to their refuges. Any such system of navigation would require special behaviours, including retinal movements, because a spider would have to survey the sky through an optical system with a very small horizontal window.

6. If our speculations so far can be taken seriously, it would seem that the dorsoventral strip of Layer IV (4a) receptive segments present in all salticids should correspond to the ventroperipheral field described by Schröer (1975, 1976). Land (1969*a*) noted that the principal optic nerve of a salticid, *Phidippus*, undergoes a 90° twist between the posterior retina and the first optic neuropile to which it projects. Unless the same twist is present in the principal optic nerves of all spiders (a matter that has never been examined) it is attractive to speculate that the salticid retina rotated through 90° during its evolution, bringing a ventroperipheral field of receptive segments into the position now occupied by Layer IV (4a).

6.4. *What determines the conformational changes to a principal retina during post-embryonic development?*

The conformational changes described in the Results stem from an interaction between only two types of cell: primordial receptor somata destined to provide the receptive segments, and primordial glial somata which differentiate to provide the matrix in which the receptive and intermediate segments are embedded. Because neither mitotic figures nor degenerating cells were observed at any post-embryonic stage, it must be assumed that the complex transformation of a ball of undifferentiated cells to an elaborate tiered retina is achieved by the differentiation of an initial population of primordial cells and interactions between them.

At first inspection, it seems likely that regulation of the morphogenesis of a principal retina may require the collaboration of more than one mechanism, an issue that is discussed in more detail in the accompanying paper (Blest & Carter 1988). The relatively late development of the glial matrix, evident even from light micrographs, suggests that much of the gross architecture of mature retinae may be driven by mechanical forces stemming from growth of the glia. The model implied is simplistic; its notional consequences can best be illustrated by the transverse sections of two principal retinae at the level of distal Layer I given in figures 50 and 51, plate 6. Figure 50 illustrates *Phidippus otiosus*, a large species from Florida with a deep prosoma. The retinal 'boomerang' is obtusely angled, and not far short of being straight. Figure 51 offers an equivalent section of a principal retina of *Ocrisiona* sp., belonging to a genus of large Australian salticids that are dorsoventrally flattened as an adaptation to their habitat under eucalypt bark. The 'boomerang' is bent into a hoop. Presumably, the dorsoventral conformations of principal retinae relate, in part, to the prosomal depths available to house them. They could be realized simply by mechanical forces generated by the differential growth of the glial matrix at the outer and inner sides of a retina.

6.5. *The diverging component of the telephoto system*

The performance of the telephoto system of the dioptrics rests upon the hemispherical interface between the contents of a retinal tube and the glial matrix. The light micrographs that illustrate this account show that the extreme proximal apex of the tube is only cleared locally of organelles during the three days that follow the moult to the second instar at day 12. By day 15, the apex of the tube is devoid of organelles, nuclei of the glass cells lie peripherally and somewhat distally, and the dense bodies that are a prominent feature of the glass cells after *ca.* day 7′ have been deleted both from the apex of the 'pit' and from the optically critical axial pathway.

We are currently examining the late morphogenesis of the diverging component in detail. Here, it is worth noting that to function effectively it must be topologically precise, and that the onus of providing an accurately shaped interface must rest largely upon late events in the differentiation of the glial matrix.

Our programme on salticid retinae started in 1979. Many collaborators have, throughout, provided stimulating comments and advice: Dr R. C. Hardie, Dr R. R. Jackson, Professor M. F. Land, F.R.S., Dr P. McIntyre and Dr D. S. Williams. All have contributed in one way or another to §§5 and 6, and none should be held responsible for the particular speculations that they contain.

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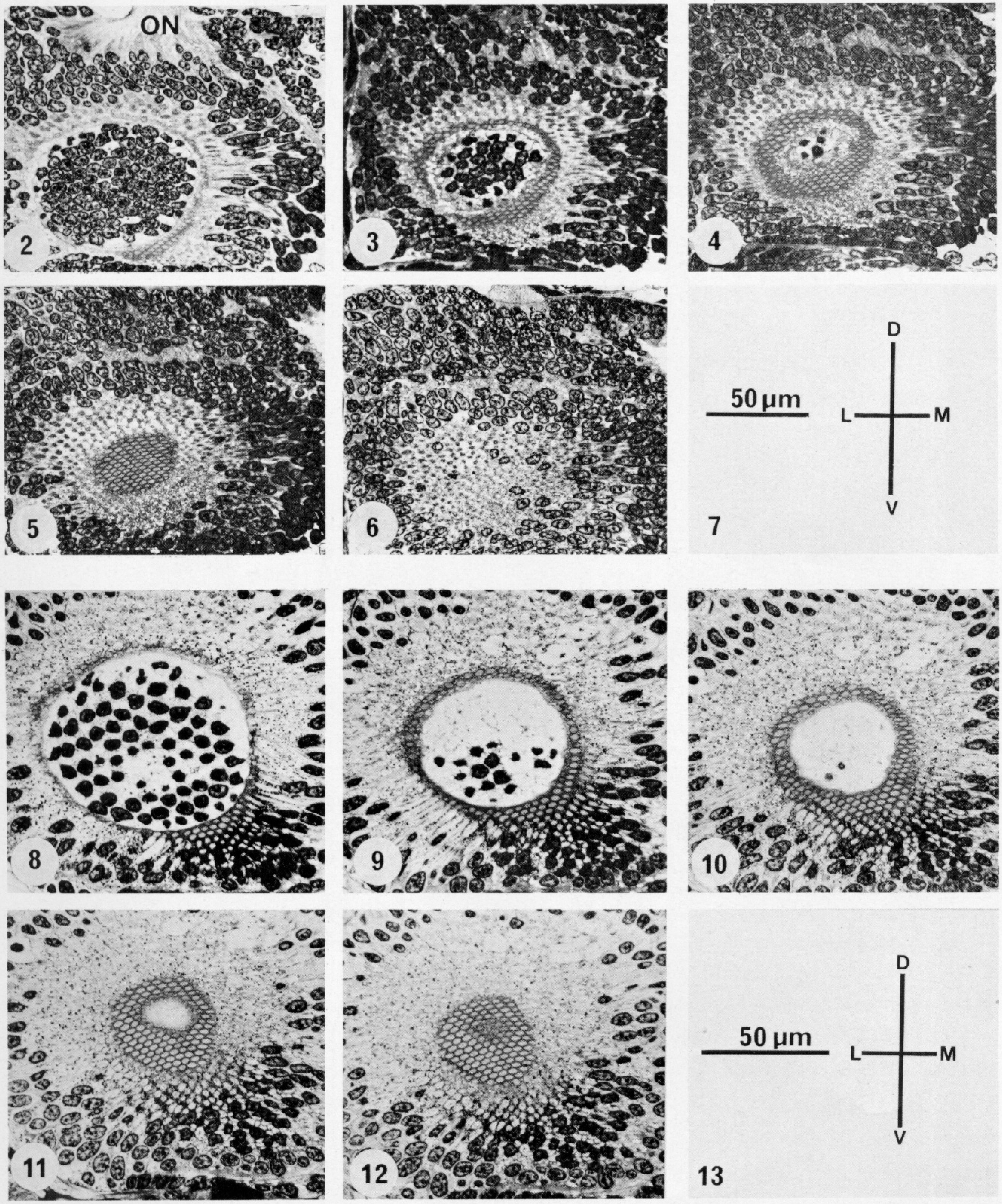
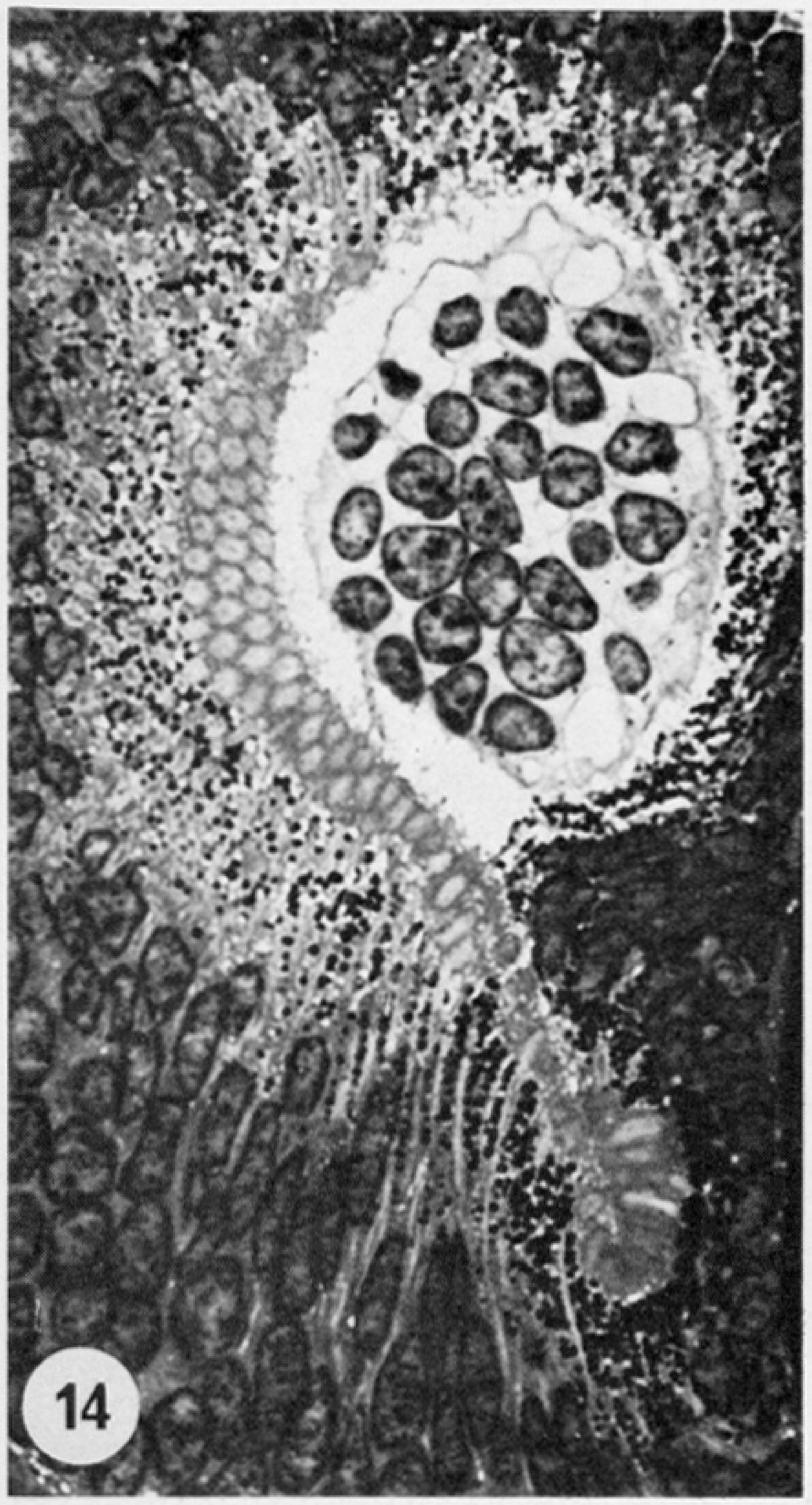
ABBREVIATIONS USED ON THE FIGURES

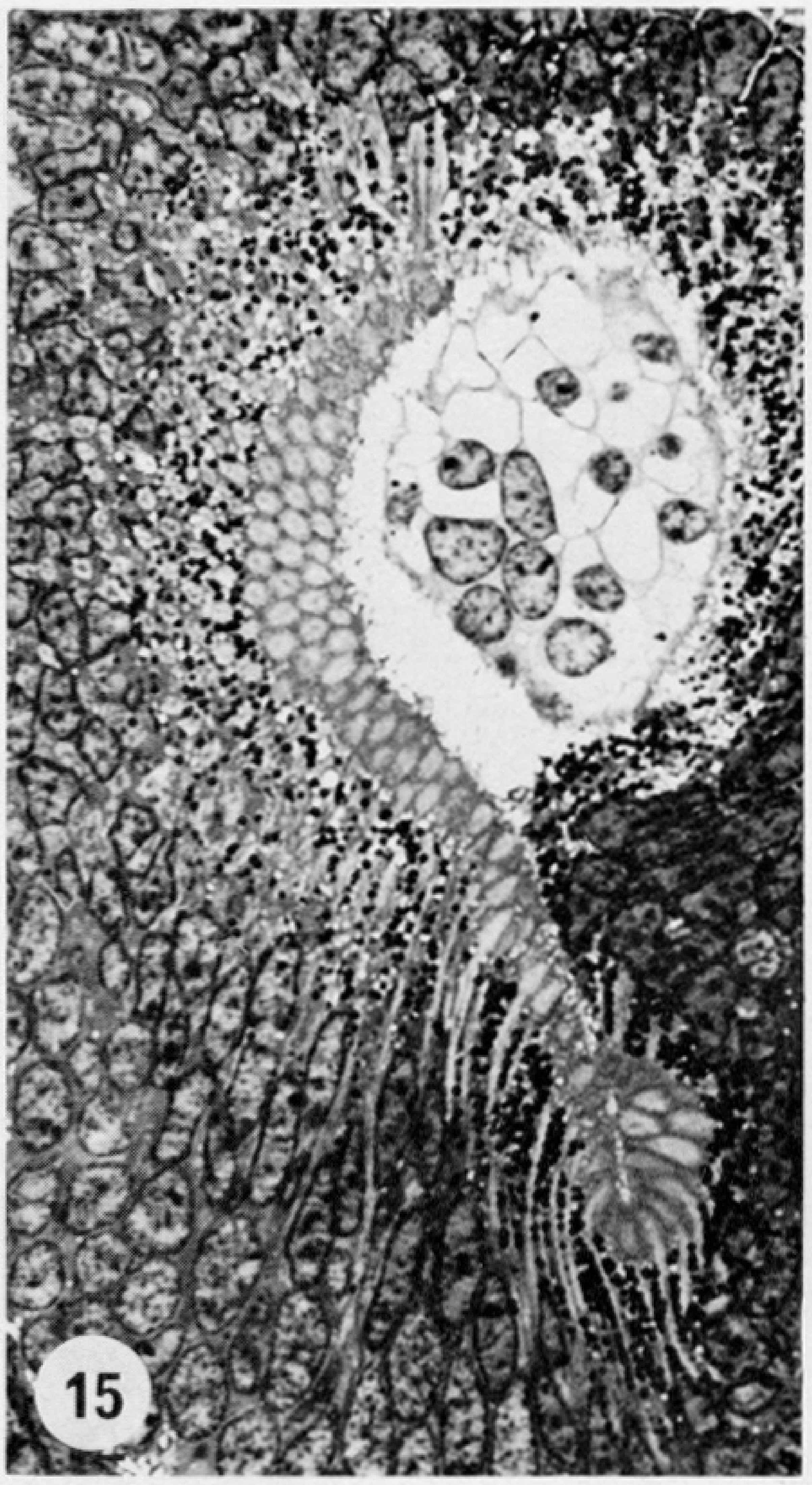
R	retina
GC	glass cells
L	corneal lens
Nu	nucleus
P	pit (the diverging component of the telephoto optical system)
DB	dense bodies in the glass cells
ON	optic nerve

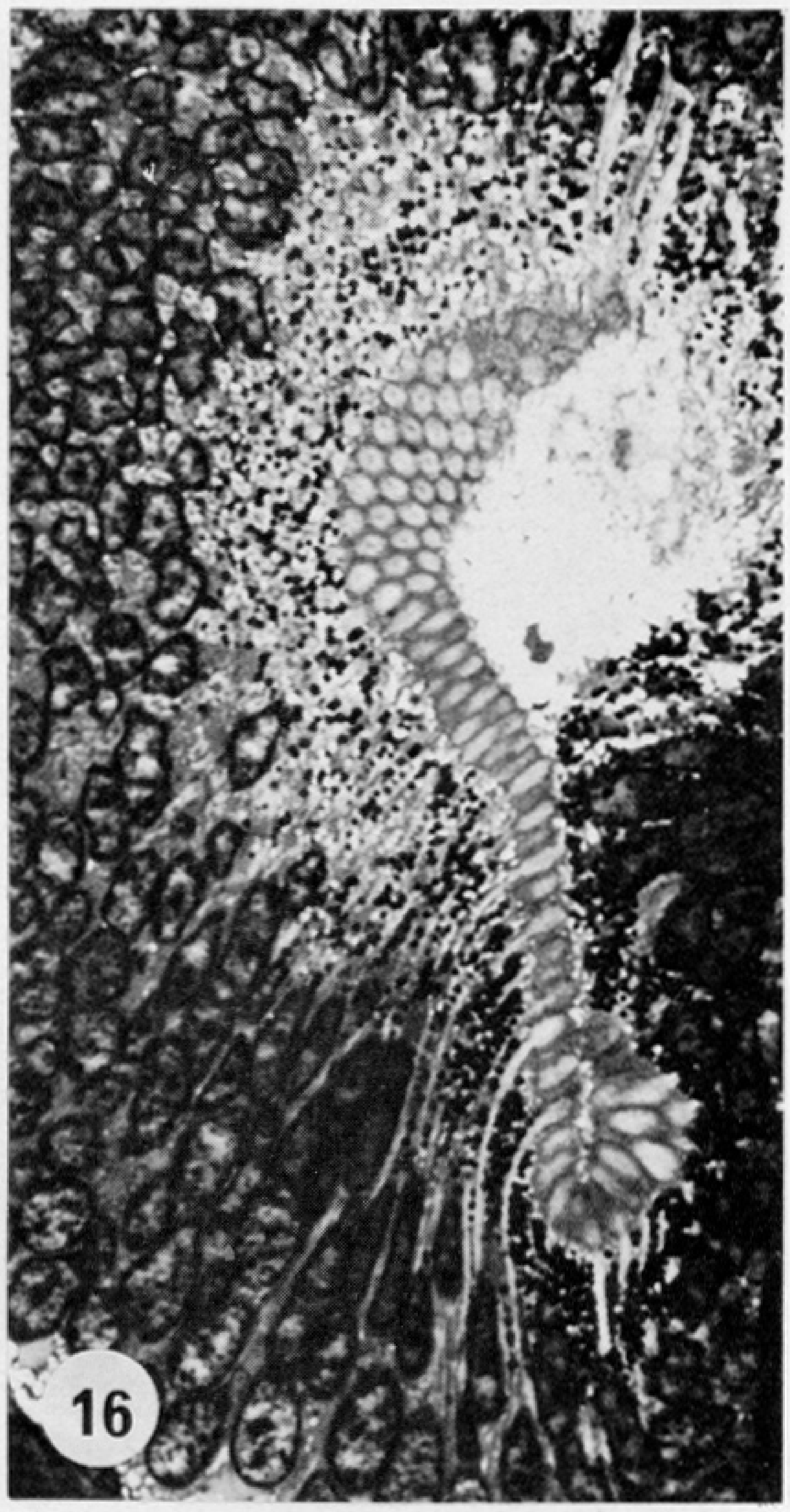
The orientations of transverse sections of retinae are indicated in plates 1–4. They are given relative to the prosoma and ignore rotational displacements of the retinae. D, dorsal; V, ventral; L, lateral (the outer side of a retina); M, medial (the inner side of a retina).

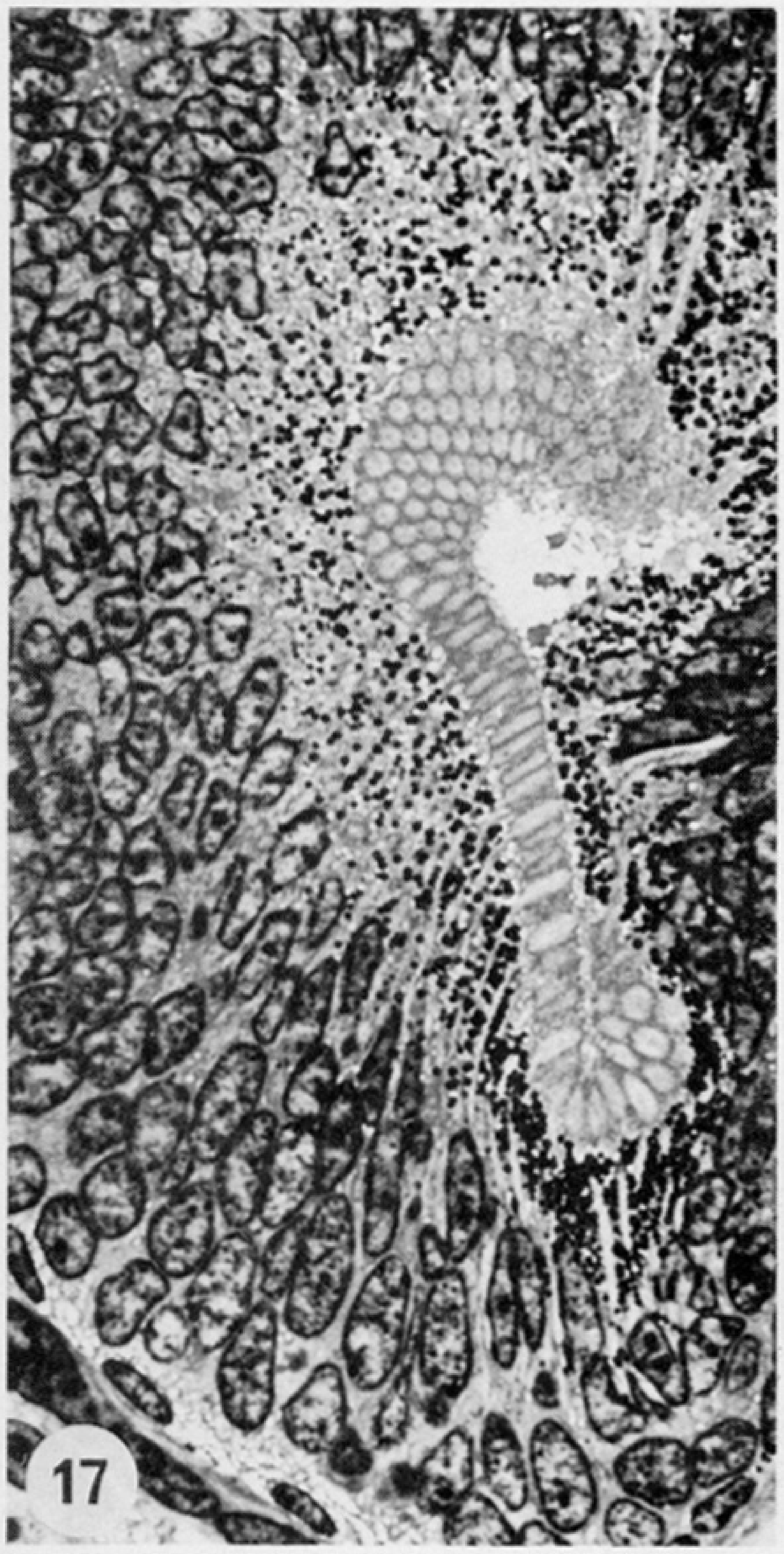


FIGURES 2–13. For description see opposite.

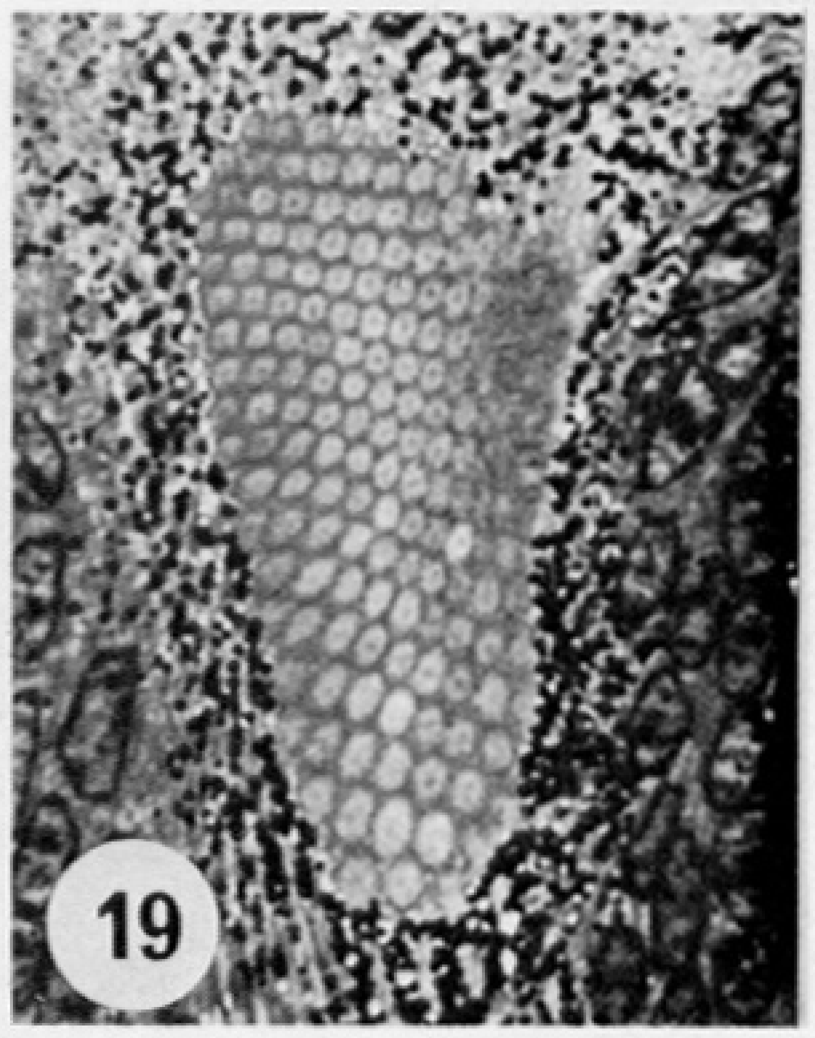

14

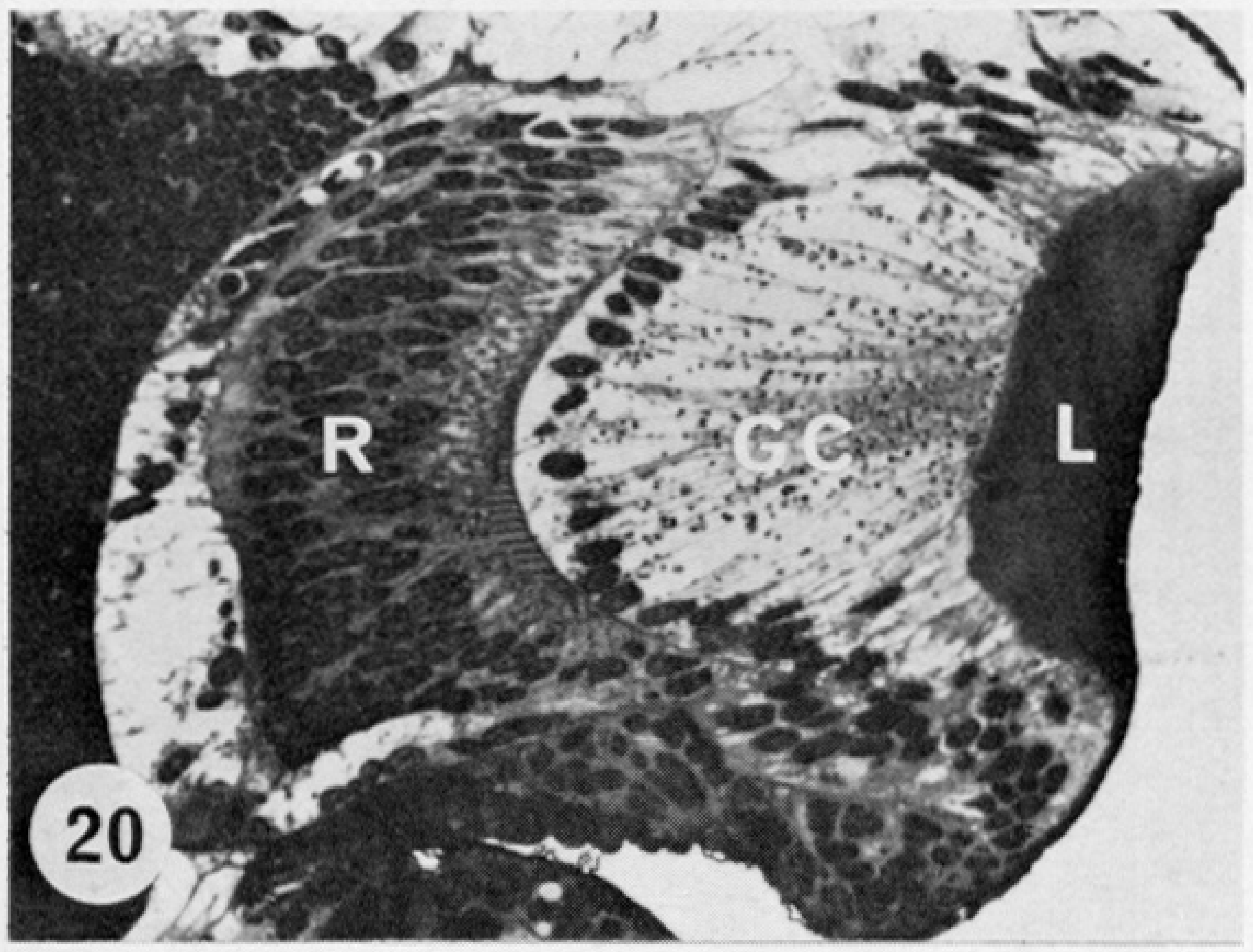

15

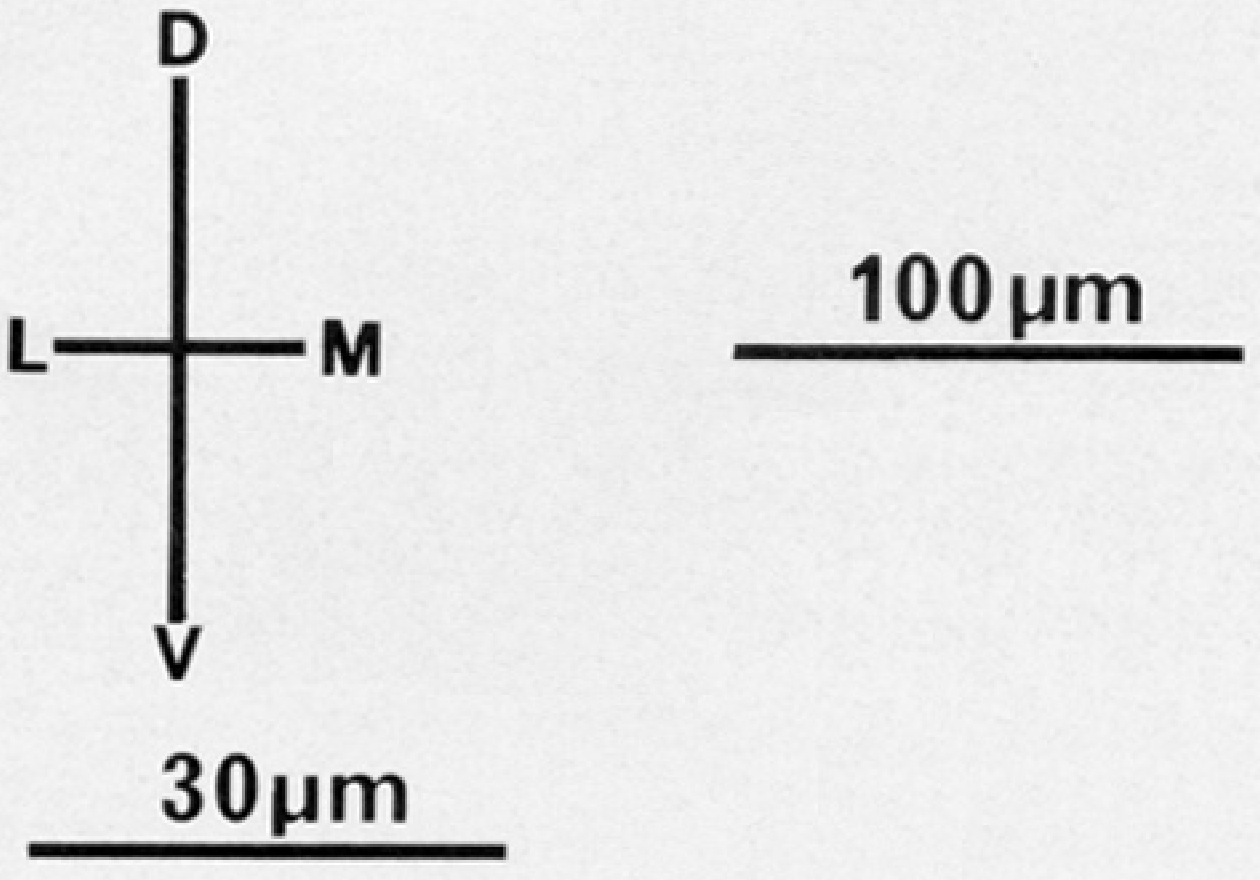

16


17

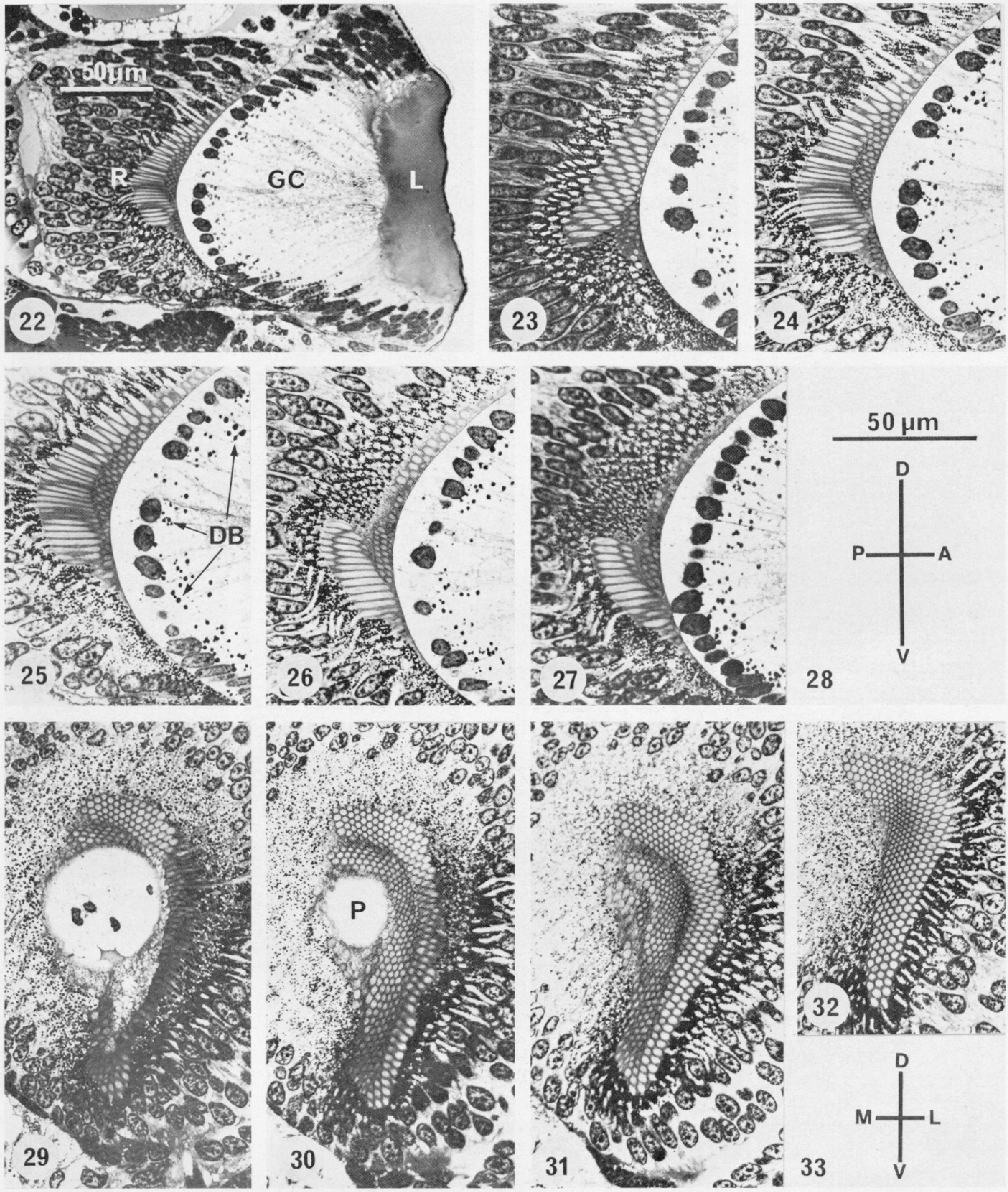

18


19

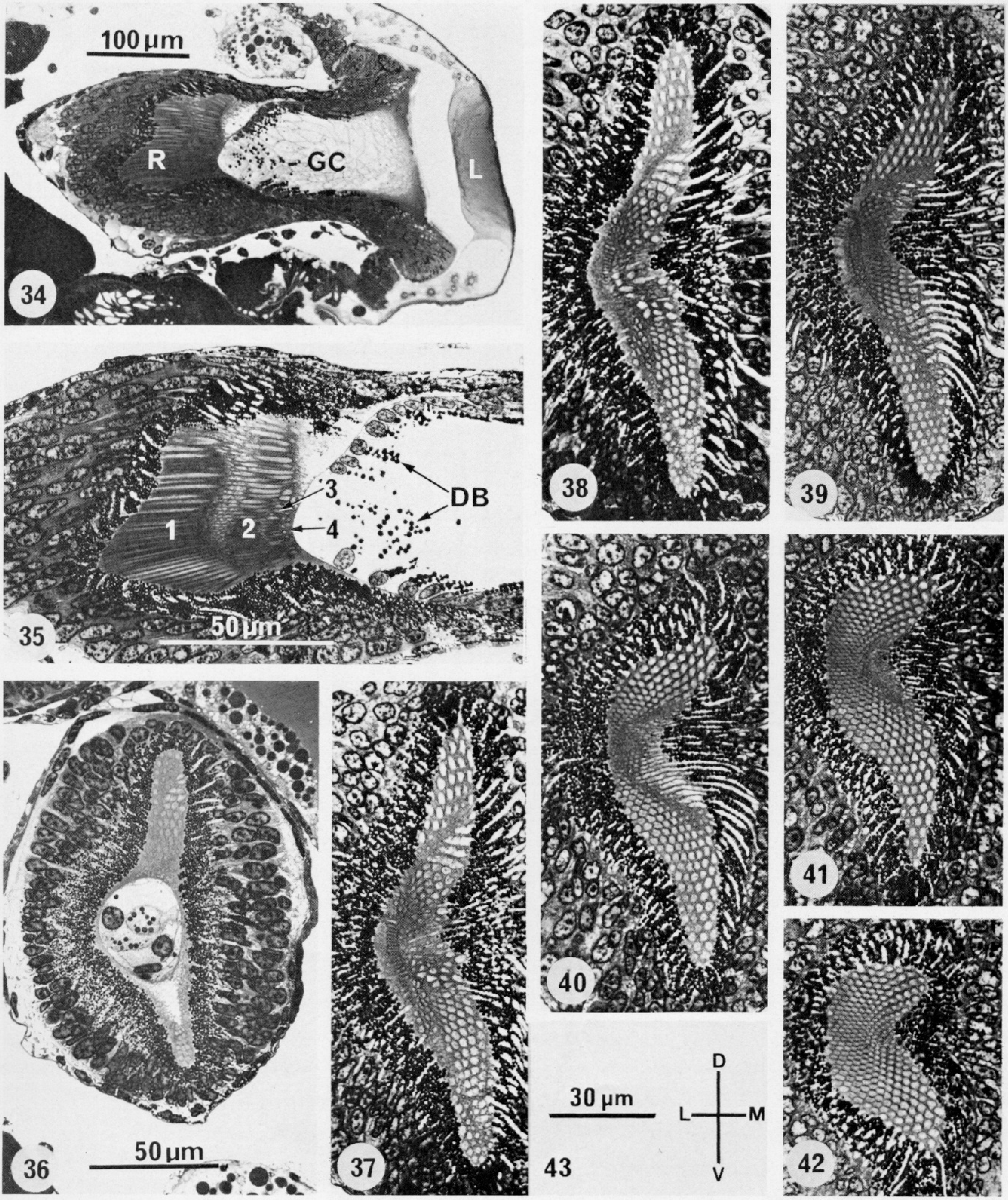

20


21

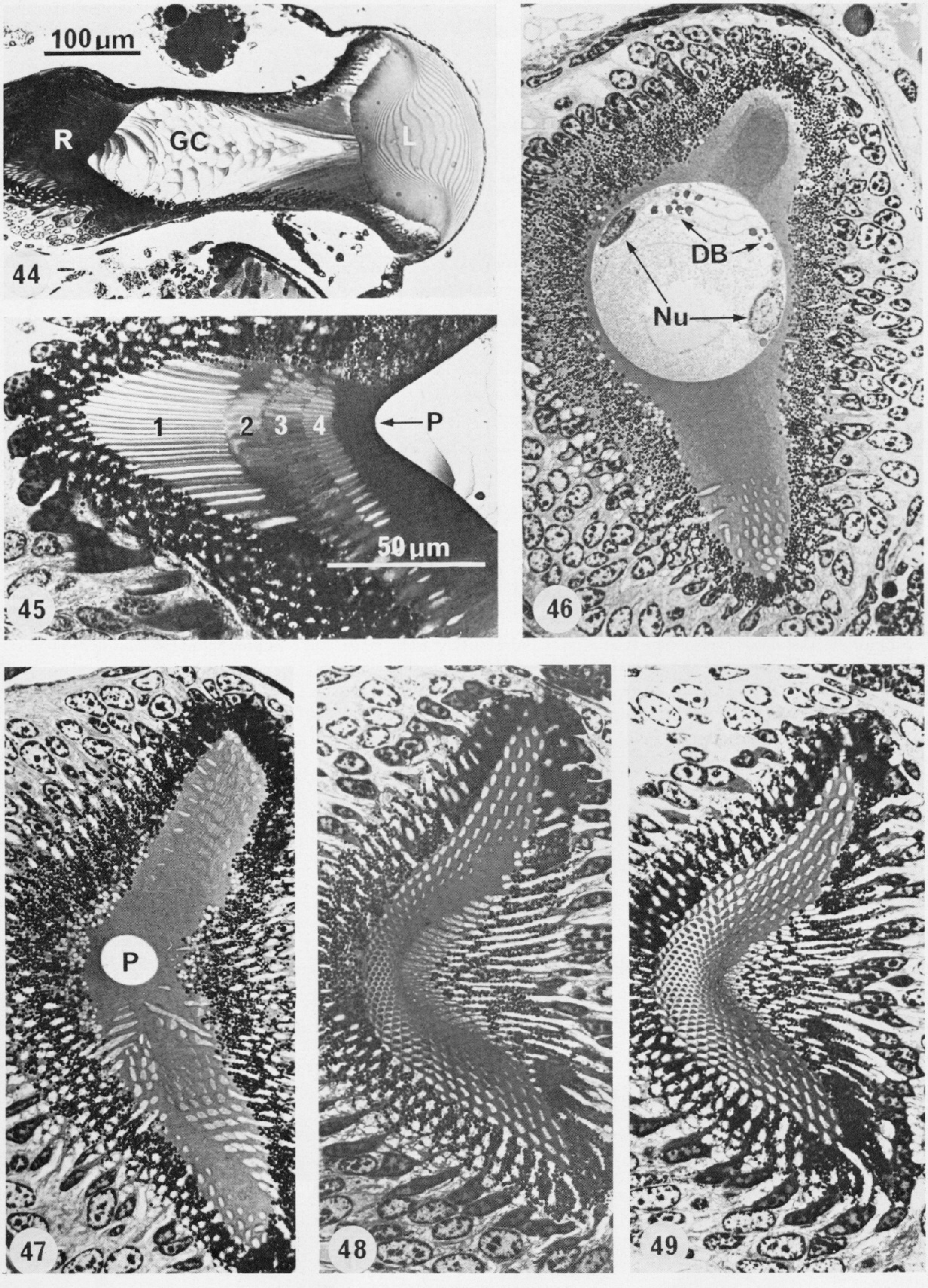
Figures 14–21. For description see facing plate 4.



FIGURES 22–33. For description see facing plate 4.

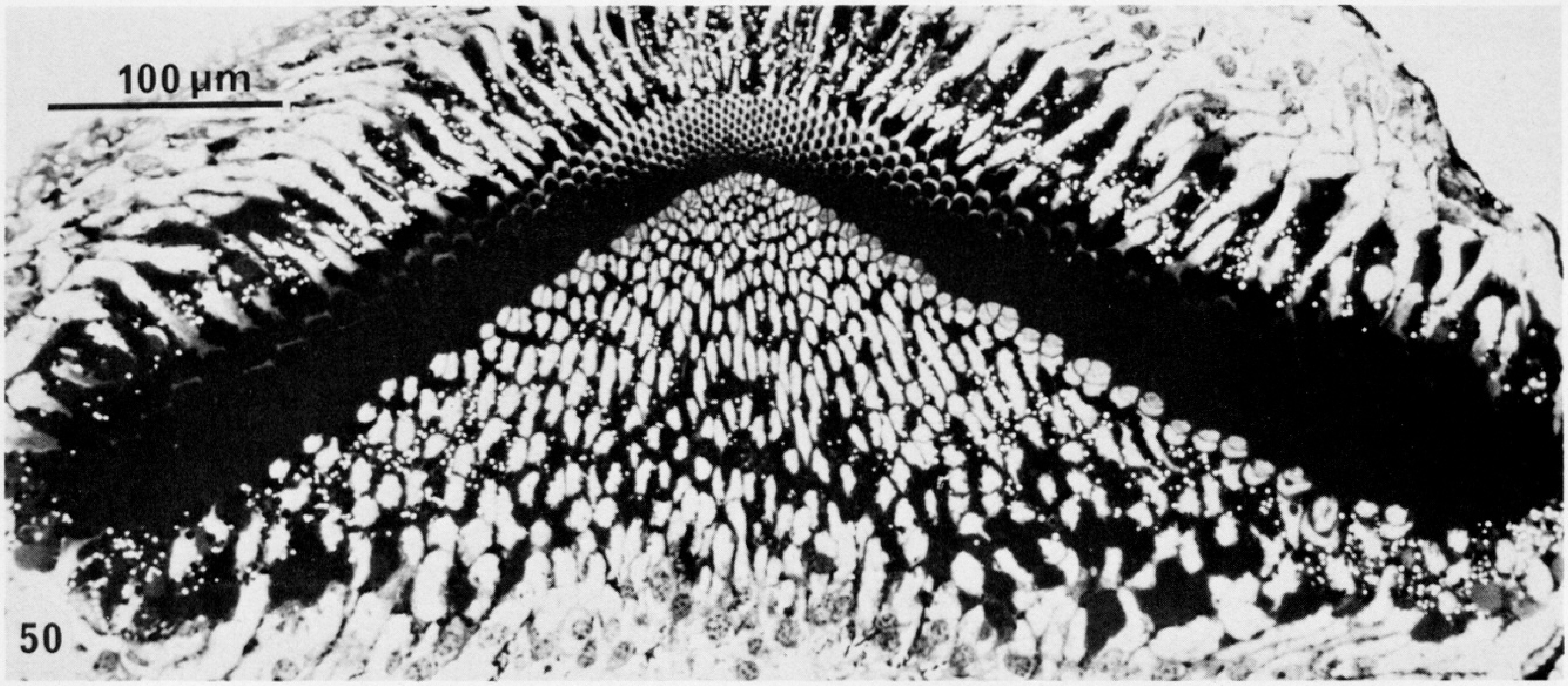


FIGURES 34–43. For description see opposite.

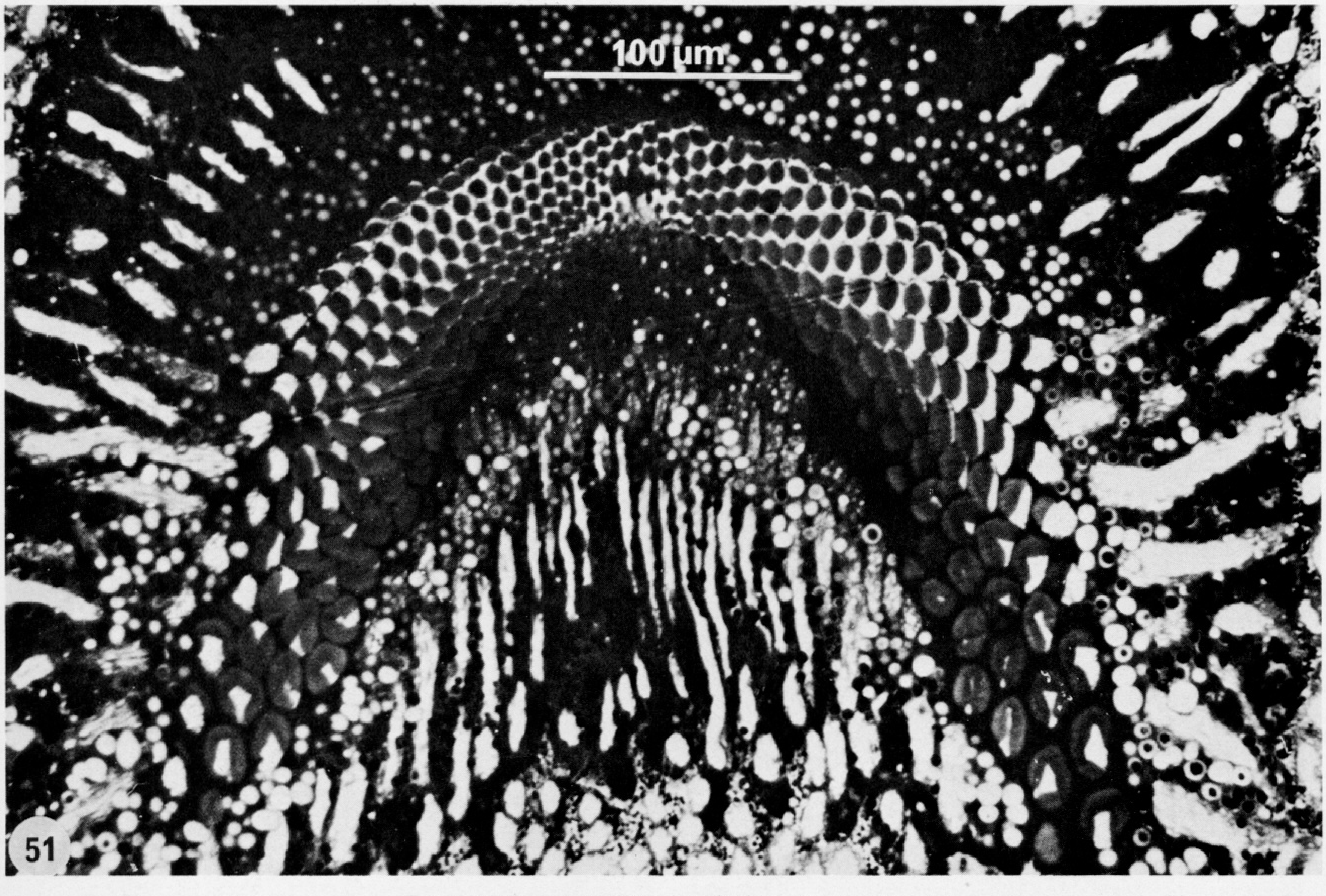


FIGURES 44–49. For description see opposite.

50



51



FIGURES 50 and 51. For description see opposite.