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STRUCTURE AND FUNCTION IN THE EXCRETORY SYSTEM OF ARCHAEOGASTROPODS AND THEIR SIGNIFICANCE IN THE EVOLUTION OF GASTROPODS

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CONTENTS

Three species of archaeogastropod mollusc, *Monodonta lineata* (da Costa), *Emarginula reticulata* Sowerby and *Patella vulgata* L. were selected as representative members of the Trochacea, Fissurellacea and Patellacea, respectively, for a comparative anatomical and ultrastructural study of the excretory system.

Primary urine formation takes place by filtration of blood through the walls of the paired auricles in *Monodonta* and *Emarginula* and of the single auricle and ventricle in *Patella*. Urine then passes to right and left kidneys along the renopericardial canals.

Contrary to earlier reports the two kidneys are different in structure and function in all three species, the larger right kidney retaining the primitive function of nitrogenous excretion, the left having a predominantly resorptive role and with a

capacity to abstract from the blood solutes of larger molecular mass. The difference in the size of the two kidneys is exaggerated in *Patella* and *Emarginula* as a consequence of partial restoration of bilateral symmetry in these limpets. It has been possible to demonstrate at the ultrastructural level that the minute left kidney of *Emarginula* is functional.

The vacuolated epithelial cells of the right kidney contain layered excretory spherules composed of purines, melanin and ferric iron in different proportions in the three genera. There is close similarity in the ultrastructural organization of these cells in *Monodonta* and *Emarginula*, but those of *Patella* show marked differences and their excretory spherules contain a higher proportion of melanin.

The position of the left kidney in the mantle skirt, as exemplified by *Monodonta*, is believed to have arisen in the earliest gastropods correlated with the development of helical coiling. This was accompanied by a change in its blood vessels. It has lost its afferent renal vein, which primitively would have carried deoxygenated blood from the viscera, an arrangement which persists in the right kidney. The left efferent renal vein is reduced in *Monodonta* and lost in *Patella* and *Emarginula*.

A new vessel has arisen linking left auricle and left kidney and there is evidence to suggest that it carries post-branchial oxygenated blood. It is believed to serve as both an afferent and major efferent route.

The physiological implications of this change in the blood supply are discussed and held to be responsible for the functional differences between the two kidneys, creating conditions in the left which favour resorption of organic solutes and ions, and leaving the right kidney with the primary role of nitrogenous excretion.

The evolution of the nephridial gland is examined in this context and is also believed to be correlated with the change in the blood supply to the left kidney. Ultrastructural evidence is given in support of its suggested resorptive function.

The significance of the differences between right and left kidneys of archaeogastropods is discussed in relation to the evolution of the monotocardian excretory system, and the possible phylogenetic relationships of the groups of archaeogastropods are considered.

Introduction

Recent ultrastructural observations on archaeogastropods (Andrews 1976, 1981; Økland 1982) are consistent with the view that the first step in urine formation is filtration of blood through the wall of the heart, the auricles being the main site of filtration. In this respect these most archaic of gastropods retain what appears to be the primitive molluscan condition, since podocytes, which indicate filtration sites, have also been identified in the auricles of chitons (Økland 1980; A. Brimble, personal communication), and some bivalves (Pirie & George 1979; Moore *et al.* 1980; Jennings 1984). Dibranchiate cephalopods are unique and specialized in having branchial hearts, the appendages of which have assumed the role of filtration (Schipp & Hevert 1981).

This leaves modification of the primary urine as the role of the kidneys. Typically in molluscs excretion of purines and other waste products and abstraction of valuable solutes are shared equally by the paired bilaterally symmetrical kidneys, different regions of these coelomoducts being specialized for specific functions.

Gastropods stand alone in departing from this plan. Right and left kidneys of all archaeogastropods show marked anatomical differences and it has been evident since the work of Perrier (1889) and Cuénot (1899) that in pleurotomariaceans and trochaceans they are also histologically and functionally different. This has been substantiated by the more recent

findings of Harrison (1962) and Delhaye (1976). The larger right kidney in these snails is responsible for nitrogenous excretion in the form of purines and has the capacity to sequester cations (Delhaye 1976), while the left kidney (papillary sac) appears to be primarily concerned with organic resorption. It has a different, probably more restricted excretory function than the right. This has been confirmed experimentally in *Haliotis* by Harrison (1962) who demonstrated secretion of phenolsulphonphthalein and para-amino hippuric acid by the right kidney but not the left. Crofts (1929) had earlier shown purine tests to be positive on the right renal epithelium and negative on the left. Traces of purines identified in the lumen of the kidney may well have originated from the pericardium which was reported by Crofts to show some excretory activity in *Haliotis*.

Further differentiation of the left kidney has occurred in trochaceans (perhaps correlated with colonization of the intertidal) in the development of the nephridial gland, which has been regarded as a possible site of ion regulation (Fretter & Graham 1962). This agrees with ultrastructural observations on the gland of the monotocardian *Littorina littorea* (L.) (Andrews 1981) and the kidney of *Viviparus* which, Andrews (1979) has proposed, is composed almost entirely of nephridial gland. Delhaye (1976) has remarked on its similarity in the trochids *Monodonta*, *Calliostoma* and *Gibbula* and in the monotocardian *Littorina*.

No trace of a nephridial gland has yet been found in any pleurotomariacean (Crofts 1929; Fretter 1964, 1966) and until one was identified in the Galapagos rift limpet *Neomphalus fretterae* McLean (Fretter *et al.* 1981) it seemed likely that it was a trochacean innovation. *Neomphalus* is on a different evolutionary line and has a habitat very different from that of trochaceans, but this suggests that some propensity for its development existed in the earliest gastropods, which may be detectable at the ultrastructural level in other extant groups of archaeogastropods.

Further examination of the left kidney of fissurellaceans and patellaceans is warranted however, on grounds more fundamental than this, in that there are major discrepancies in accounts of its organization. Delhaye (1976) agreed with earlier descriptions that in both groups right and left kidneys are identical at the histological level despite the profound differences in their anatomy. This has been disputed by Andrews (1981) and clarification is central to an understanding of the excretory system of archaeogastropods in phylogenetic, physiological and ecological terms. The evolutionary mosaic implied by the earlier reports has up till now eluded rational explanation.

Furthermore, questions still remain as to how anatomical, as distinct from mere size differences arose in the kidneys of archaeogastropods in the first instance and how they are correlated with functional differences known to exist at least in pleurotomariaceans and trochaceans. If these distinctions between the kidneys are demonstrated in all four groups discussed here then they probably arose in the ancestral gastropods and the factors responsible are likely to have been basic in the evolution of the class.

Inferences based on knowledge of renal organization in different groups of archaeogastropods moreover, might help understanding of phylogenetic and functional problems in monotocardians, now generally accepted as being polyphyletic. In monotocardians and their descendants it is always the left kidney that persists as the functional excretory organ. Possession of a nephridial gland, on the other hand, is by no means universal. A clearer understanding of renal organization in archaeogastropods might therefore assist in interpretation of the monotocardian condition.

A comprehensive answer to the questions posed above should properly contain three elements: the comparative anatomy of the excretory system, a survey of the ultrastructure, and experimental evidence. Only the first two aspects fall within the scope of this paper. As yet, Harrison's physiological study of the kidneys of *Haliotis* (1962) stands alone in using modern experimental techniques to investigate mechanisms of urine formation in an archaeogastropod.

Most of the anatomical background is already known and needs amplification here only in relation to the left kidney in *Emarginula* and *Patella* and its blood supply in *Monodonta*. Earlier accounts such as those by Davis & Fleure (1903) of *Patella*, Crofts (1929) of *Haliotis* and Nisbet (1953) (among others) of *Monodonta*, have already been reviewed and expanded with original observations by Fretter & Graham (1962). Subsequently Fretter (1964, 1966) has examined the pleurotomariaceans *Mikadotrochus amabilis* Bayer and *Perotrochus*.

Materials and methods

The same method of fixation for electron microscopy was used for all species studied, namely *Emarginula reticulata* Sowerby from Millport, *Patella vulgata* L. collected in Aberporth, Dyfed, *Gibbula cineraria* (L.), *Monodonta lineata* (da Costa) and *Calliostoma zizyphinum* (L.) from Plymouth. The primary fixative used was glutaraldehyde (50 g l^{-1}) in 0.1 M Sorensen's phosphate buffer pH 7.2 containing 0.479 M (140 g l^{-1}) sucrose. Post-fixation in osmium tetroxide (10 g l^{-1}) in the same buffer was followed by embedding in TAAB resin. Sections were stained in aqueous uranyl acetate and Reynolds' lead citrate. Anatomical observations were made on live animals dissected in sea water.

The PAS test, using diastase on control sections, was used to confirm the presence of glycogen in the podocytes of *Emarginula*.

Results and discussion

Anatomy of the excretory system and associated blood vessels

No archaeogastropods described here can be regarded as primitive: indeed the fissurellaceans and patellaceans are far removed from the ancestral form probably best reflected in pleurotomariaceans, specimens of which were unobtainable. Trochids, however, have enough in common with pleurotomariaceans to suggest close relationship (Salvini-Plawen 1980) and are adopted as a reference group with which the others are compared.

Filtration site

The structure of the paired auricles, believed to be the major site of filtration in *Monodonta* and other trochids has already been described (Andrews 1976*b*, 1981). Their most significant feature is the pouches (figure 1, fp) which can be isolated by muscles from the main lumen, an arrangement that suggests a mechanism for regulating the relative proportions of blood for systemic circulation and filtration.

The bilateral symmetry of the auricles in *Emarginula* (figure 2, lau, rau) contrasts with their asymmetry in *Monodonta* in which the right auricle is smaller than the left, and in *Patella* in which it is lost. The two rows of filtration pouches typical of *Haliotis* as well as *Monodonta* (Andrews 1981) are absent from both, perhaps as a consequence of small size in *Emarginula*, and correlated with the development of a porous epicardium over the entire surface of the highly modified heart in *Patella* (figure 3).

In *Emarginula* a bay of each auricle close to the openings of the veins is specialized for filtration, being covered by podocytes, and is demarcated by muscle fibres from the main chamber which is covered by a continuous squamous epithelium. It appears therefore that in this animal too, the filtration chamber can be isolated.

In *Patella* this is not possible since both auricle and ventricle are completely covered by podocytes (Økland 1982).

The kidneys

The two kidneys of the archaeogastropods described here differ in position, size, ratio of epithelial surface area to volume of lumen, size and rhythm of opening of renopericardial canal, and extent of subepithelial musculature. The differences are extreme in fissurellaceans and in patellaceans in which the adoption of a limpet shape has resulted in the left kidney of an originally helically coiled form being reduced because the growth rate of the left is no longer relatively greater than the right. This is accompanied by the virtual loss of the post-branchial region of a pleurotomariacean, adding another limiting factor to the size of the left kidney. In patellaceans some asymmetry persists in the pallial complex and the left kidney is displaced to the right. Complete bilateral symmetry is secondarily restored in the pallial complex of fissurellaceans with the result that the left kidney survives only as a minute vesicle in the posterior wall of the mantle cavity (figure 2, lk). While the right kidney is consistently the more capacious throughout the archaeogastropods it does not appear to offer as effective a surface area for exchange of solutes between urine and epithelium as the left kidney: its renopericardial canal is smaller and the generally shorter cilia may not create such rapid flow of fluid over the epithelium, though evidence on rates of flow of primary urine is limited. Furthermore, the restricted area of excretory folds on its walls, coupled with a large capacity suggests that it is essentially a vehicle to receive nitrogenous waste and functions to some extent as a body cavity (Andrews 1981). The conditions in the left kidney, by contrast, appear to favour rapid modification of relatively small amounts of primary urine virtually uncontaminated by nitrogenous waste.

The additional role of the right kidney as a passage for gametes is typically associated only with its most distal part in which gametes may be virtually isolated from the urine. A strongly ciliated tract free of excretory activity provides a distinct pathway to the kidney opening.

These features are exemplified by *Monodonta* (figure 1). The capacious right kidney embedded in the visceral mass is subdivided into two distinct regions: a large proximal part (prk) consisting of a visceral coelom posterior to the pericardium, and a smaller tubular distal part (drk) in the mantle skirt to the right of the rectum. The afferent renal vein enters the proximal lobe ventrally on the right close to the origin of the distal part, branching repeatedly over the posterior wall which is a spongy mass of tubular evaginations of the kidney lined by excretory epithelium. It is this area that abuts the digestive gland and is the major site of excretory activity. Elsewhere the walls are smooth. The opening of the gonadial duct (go) is on the right of the posterior wall close to the renopericardial opening. The strongly ciliated groove (first identified by V. Fretter, personal communication) extending from the gonadial to the external kidney opening is the only part of the kidney over which the epithelium is not excretory, and it appears to provide a route for gametes. All ciliary currents are directed towards the kidney opening.

The more compact left kidney (papillary sac, lk) has no role in nitrogenous excretion. Its walls are more muscular than those of the right, and contractions observed in live animals

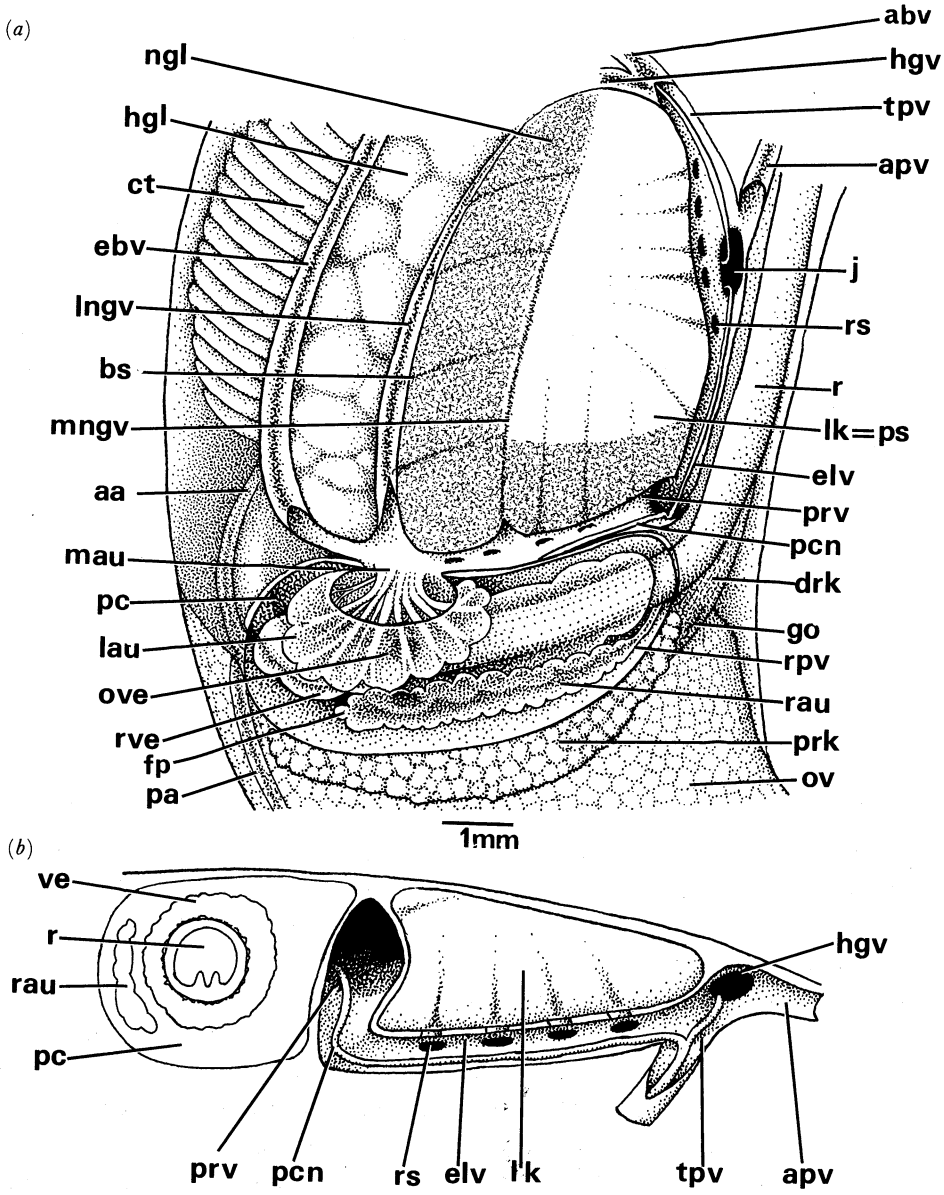

FIGURE 1. (*a*) Dorsal view of the posterior region of the mantle skirt and base of visceral hump in *Monodonta lineata*. (*b*) Diagrammatic longitudinal section along the right margin of the left kidney. aa, Anterior aorta; abv, afferent branchial vein; apv, anterior pallial vein; bs, blood space in nephridial gland; ct, ctenidium; drk, distal part of right kidney; ebv, efferent branchial vein; elv, left efferent renal vein; fp, filtration pouch; go, opening of gonadial duct into right kidney; hgl, hypobranchial gland; hgv, hypobranchial gland vein; j, junction between efferent renal vein and transverse pallial vein; lau, left auricle; lk, left kidney; lngv, left vein of nephridial gland; mau, origin of auricular muscles; mngv, median vein of nephridial gland; ngl, nephridial gland; ov, ovary; ove, auricular–ventricular opening; pa, posterior aorta; pc, pericardial cavity; pcn, pericardial nerve; prk, proximal part of right kidney; prv, posterior renal vein; ps, papillary sac; r, rectum; rau, right auricle; rpv, right pallial vein; rs, renal blood space; rve, rectum surrounded by ventricle; tpv, transverse pallial vein; ve, ventricle.

removed from their shells may normally be more important than the cilia in mixing the urine. Fluid injected into the lumen of the kidney induces strong rhythmic contractions of transverse and longitudinal muscles, though mechanical stimulation of the dorsal wall evokes only a few local contractions. In its superficial position in the mantle skirt independent muscular activity of the papillary sac is more feasible than in the right kidney. A nephridial gland (ngl) occurs (Fretter & Graham 1962), in this respect an advance over pleurotomariacean organization. The gland is L-shaped, extending along the posterior (pericardial) and left walls of the kidney, being most pronounced along the pericardium. Subepithelial muscles traverse the gland and contract independently of the network over the rest of the dorsal wall.

The papillary sac is highly vascular throughout, most markedly in the nephridial gland, and is intimately connected with the left efferent ctenidial vein (ebv) and left auricle (lau). While there are connections between the blood spaces of the nephridial gland and those in the rest of the kidney the arrangement of vessels appears to allow virtually independent and relatively rapid circulation through the gland, directly influenced by heart beat. The left strip of the gland is bordered on each side by a large vein: that on the left (lngv) connects with the left efferent ctenidial vein (ebv) at the mouth of the auricle while that on the right (the medial nephridial gland vein, mngv) communicates with a transverse vein (prv) in the pericardial wall, not previously described. This runs along the posterior margin of the kidney and at its left end opens into the auricle. The gland is traversed by branches of the veins on its margins some of which form direct cross-connections.

No valves control blood flow at the auricular opening but cardiac muscles originating in the wall of the veins (mau) are so oriented that they appear on contraction to occlude the opening of the left vein of the nephridial gland into the auricle, though not its junction with the ctenidial vein. Despite the present lack of other evidence the anatomical arrangement strongly suggests that post-branchial blood flows into the nephridial gland vein from the ctenidial vein at auricular systole and back to the auricle via the transverse vessel at diastole. This posterior renal vein is difficult to delimit from the auricle and is clearly a major connection between auricle and all parts of the papillary sac. Indeed some renal sinuses open directly into the auricle. At the right corner of the pericardium, limited on one side by the sac and on the other by the rectum, the vein appears to end, but, after giving off a small branch to the right margin of the kidney it dives ventrally, turning through almost 90° and again through another near right angle to extend along the deeper parts of the right wall of the papillary sac, from which it receives branches (figure 1*b*, rs). This section finally opens into the transverse pallial vein (tpv) and is identified at this point by Fretter & Graham (1962, figure 145) as an efferent vein of the kidney. It is nearly circular in cross section but the part bordering the pericardium is triangular, its anterior wall stiffened by collagen fibres, the posterior thin and easily compressed by rising pericardial pressure or dilated by internal blood pressure. Both this deformability and the double bend in the vein may help to regulate flow along it. The cylindrical shape of the pallial section (clearly the homologue of the left efferent renal vein of a pleurotomariacean), suggests that it remains permanently open.

It is difficult to identify the nephridial gland on the inner surface of the papillary sac since, like the rest of the wall, it bears papillae, albeit shorter and fewer than elsewhere. It is thick, with a spongy texture due to many pit-like invaginations among the bases of papillae, whereas the right wall is thin and smooth over the efferent renal vein.

In *Emarginula* (figure 2) the bilateral symmetry of the pallial complex and heart does not

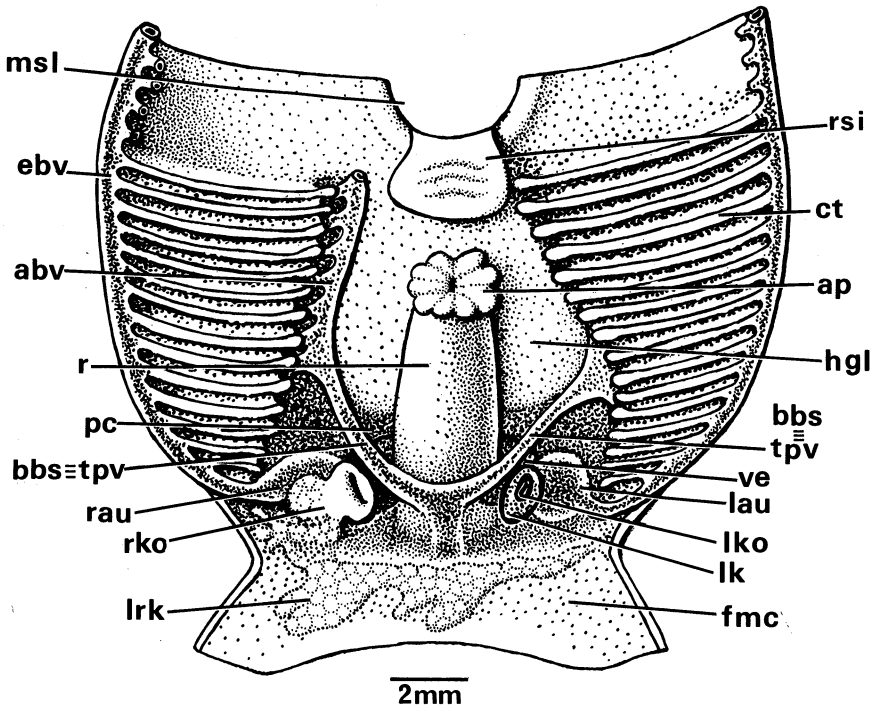

FIGURE 2. Dissection of the posterior part of the mantle cavity of *Emarginula reticulata*, the mantle roof lies uppermost in the figure, the floor below. abv, Afferent branchial vein; ap, anal papilla; bbs = tpv, basibranchial sinus homologous with transverse pallial vein; ct, ctenidium; ebv, efferent branchial vein; fmc, floor of mantle cavity; hgl, hypobranchial gland; lau left auricle; lk, left kidney; lko, left kidney opening; lrk, lobe of right kidney; msl, slit in mantle skirt; pc, pericardial cavity; r, rectum; rau, right auricle; rko, right kidney opening; rsi, retracted siphon; ve, ventricle.

extend to the kidneys which show a striking difference in size. In this animal, the right kidney constitutes an even more extensive and pervasive body cavity than in *Monodonta*. It lacks the tubular extension of the kidney into the mantle cavity typical of *Monodonta* and pleurotomariaceans, probably because the post-branchial region of the cavity is lost. There is nevertheless a wide smooth-walled distal part leading to the external opening and bearing a ciliated tract linking the external opening (rko) to the gonadial duct on the right. This appears to be the counterpart of the tubular section of *Monodonta* subserving the same function of conveying gametes. In *Emarginula* the epithelium of this region is not excretory. The right renopericardial canal (rpc) opens on the left wall of the distal part.

The excretory part of the kidney is elaborately lobed, part of it extending ventral and posterior to the pericardium to emerge dorsally on the left side where it creates the misleading impression of a left kidney, possibly responsible for misidentification in earlier accounts. The latter is reduced to a minute vestige lying against the anterior wall of the pericardium. The blood supply of the right kidney follows the typical archaeogastropod pattern.

The minute vesicle that constitutes the left kidney of *Emarginula* is no more than 300 μm in diameter and appears as a translucent ovoid pocket (figure 2, lk) in the posterior wall of the mantle cavity. Its slit-like external opening (lko), borne on a small papilla left of the rectum has occasionally been seen to open, but with neither obvious rhythm nor synchronization with opening of the right kidney nor with heart beat. Many branches from a visceral nerve innervate

radial muscles in the lips of the opening. A renopericardial canal is traceable only in sections. Its pericardial opening is close to the left auricle and its renal opening is on the left wall of the vesicle. A renal vein running parallel to the canal links the kidney with the left efferent ctenidial vein where it opens into the auricle as in *Monodonta*. There is no detectable efferent renal vein but on its right margin the kidney directly abuts the basibranchial sinus (bbs) so bearing the same relationship with this major route as a papillary sac.

The secondary bilateral symmetry of *Patella*, although less perfect than in *Emarginula* (figure 32*c*) has also led to the withdrawal of the kidneys into the visceral hump, and the development of more asymmetry in the two organs than in *Monodonta*.

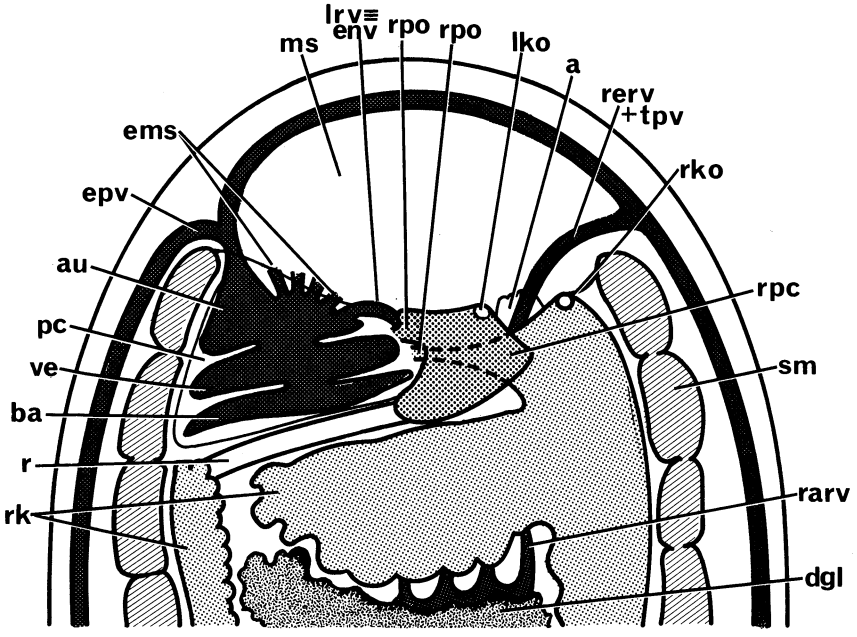

FIGURE 3. Diagrammatic representation of the renopericardial complex in *Patella* in dorsal view. That portion of the rectum which lies ventral to the left kidney is omitted. a, Anus; au., auricle; ba, bulbus aortae; dgl, digestive gland; ems, efferent veins from mantle skirt; env, efferent vein of nephridial gland; epv, efferent pallial vein; lko, left kidney opening; lrv, single vein of left kidney; ms, mantle skirt; pc, pericardial cavity; r, rectum; rarv, afferent renal veins to right kidney; rerv, right efferent renal vein; rk, lobes of right kidney (ventral lobe omitted); rko, right kidney opening; rpc, right renopericardial canal opening into subrectal lobe; rpo, renopericardial opening; sm, shell muscle; tpv, transverse pallial vein; ve, ventricle.

The right kidney of *Patella* (figure 3, rk) has a greatly enlarged proximal region lined by excretory epithelium and a small distal region with ciliated epithelium. The renal papilla (rko) projects only a short distance into the mantle cavity.

The lobed proximal part is divisible into a dorsal folded portion behind the pericardium and a smooth-walled, mainly ventral portion, some lobes of which spread on to the dorsal surface. The branches of the afferent renal vein (rarv) produce trabeculae in the postpericardial lobe which reduce the lumen to a small and much subdivided space, but ventrally it is uninterrupted and capacious.

The small renopericardial canal (rpc) opens from the right margin of the pericardium into a small lobe of the proximal region beneath the rectum, close to the renal papilla in the same relative position as in *Monodonta*. Since ciliary currents are directed towards the kidney opening

muscular activity is believed to be responsible for circulation of primary urine in the posterior and ventral lobes which are remote from the canal.

A pit-like invagination in the dorsal wall of the ventral lobe is believed to be the site of a temporary gonadial opening which may appear on the rupture of the exceptionally thin wall at the base of the pit when the gonad is ripe. This is consistent with the views of Davis & Fleure (1903). Contraction of the shell muscle may assist ciliary currents in conveying gametes towards the kidney opening, though this possibility was dismissed by Davis & Fleure.

The small size of the renopericardial canal relative to that of the kidney suggests that although only a small volume of urine enters the kidney at any one time it may be stored for relatively long periods, acting as a reserve of fluid.

The left kidney is less markedly reduced than in *Emarginula*, and is totally different in organisation. In contrast to the uniformly thin-walled vesicle of *Emarginula*, this structure is thin-walled only where it lies against the rectum; on the pericardial side it is thick and spongy, with tubular invaginations reminiscent of a nephridial gland.

The suggestion of Davis & Fleure (1903) that there might be a direct connection between the kidney vessels and auricle has been confirmed by dissection and serial sections. The renal vein (lrv) is the last of a series opening separately into the auricle, the others returning blood from the mantle skirt (ems). The vessel does not connect with the extensive subepithelial blood spaces normally found in a nephridial gland but instead fragments into very small spaces surrounded by dense connective tissue and muscle fibres (figure 16, plate 5, mf). This does not seem consistent with Davis & Fleure's description of 'numerous blood lacunae' in the heterogeneous mass that constitutes the thickened wall between the kidney and pericardium. No connection has been found between the perivisceral sinus and left kidney; the efferent renal vein has become vestigial or been lost, so that the connection between auricle and kidney must form a two-way route, as in other archaeogastropods.

Ultrastructure

The site of filtration

It has already been established that the epicardium of the heart in trochids and *Patella* is composed of podocytes, which are taken to be indicative of filtration (Andrews 1976*b*, 1981; Økland 1982). In both the podocytes are squamous-cuboid cells which have cytoplasm with few organelles and showing little sign of activity.

Part of the epicardium of *Emarginula*, that overlying the bay of the auricle, is composed of podocytes, as is the pericardium over the veins. There is a gradual transition from squamous cells to podocytes which are much taller than those of *Monodonta* and *Patella* (figure 4, plate 1, po). They are also unusual in carrying large deposits of glycogen (figure 5, gly) in the perinuclear cytoplasm suggesting that they may have assumed the role of resorbing glucose from the primary urine as a consequence of the reduction of the left kidney, which subserves this function in *Haliotis* (Harrison 1962). Adjacent cells also show a most unusual arrangement of pedicel-like connections (rpe) at points far above the basal lamina (plate 1). They appear to isolate intercellular spaces (ics) from pericardial cavity, and like normal pedicels bear diaphragms. The latter are atypical of gastropods in that teeth on opposite sides of the filtration slit are linked by a central zig-zag filament (figure 7 inset, d). The whole structure forms a relatively coarse grid of one layer, not two as in other gastropods, and is very similar to that described in a number of bivalves (Jennings 1984). The development of 'raised pedicels' may

be correlated with the height of the podocytes, which probably require greater structural support than the squamous-cuboidal ones of other species. Unusually large intercellular junctions (figure 6, j) also occur, in which there appear to be broad cytoplasmic bridges, and these may contribute both to support and intercellular communication.

The kidneys

Ultrastructural observations establish that the right and left kidneys of *Emarginula* and *Patella* are markedly different at the cellular level as they are in *Monodonta*. There is thus a consistent pattern in archaeogastropods: the right kidney retains its primitive excretory function and the left is predominantly concerned with resorption of solutes. This latter, more recently acquired, function is developed to a different extent in the three genera described here with greater resultant ultrastructural variation than is shown by the right kidney. However, the epithelium of both kidneys shows features associated with active transport: the apical surface bears both cilia and microvilli, the cytoplasm contains profiles of coated pits and vesicles apically and basally, and the basal surface area is increased by branching.

The major difference lies in the possession of an elaborate vacuolar system in the epithelium of the right kidney in which nitrogenous waste, lipofuscins, melanins and ferric iron are the most important constituents of the excretory granules (Delhaye 1976), and the prominence of secondary lysosomes and peroxisomes in that of the left.

The right kidney

The major ultrastructural features of the excretory cells in the three genera have already been briefly described by Andrews (1981) but this account needs amplification.

Their excretory cells are multivacuolated and it was suggested that the vacuoles are derived from smooth endoplasmic reticulum.

This interpretation may not be tenable in the light of recent work (Farquhar & Palade 1981) which suggests that cell membranes fall into two types: inner, comprising the nuclear membrane and endoplasmic reticulum, and outer, comprising the plasmalemma and its derivatives together with secretory vesicles budded from the concave face of the Golgi body. Vesicles bounded by outer membrane cannot fuse with inner membrane and so never open into endoplasmic reticulum. Since vesicles which appear in electron micrographs to originate by endopinocytosis fuse with excretory vacuoles the vacuolar membrane could not, according to the view expressed above, be derived from endoplasmic reticulum, and an alternative possibility is its origin in lysosomal membrane. Frequently, sections show vacuoles with a core of lipofuscin (plate 2, lc) (normally contained in lysosomes), also noted by Delhaye (1976). This suggests that lysosomes are involved in the early stages of excretory spherule formation, providing a nucleus on which other materials precipitate. If the lysosomal membrane is added to by fusion with pinocytotic vesicles an excretory vacuole of increasing diameter will be created. Spherules are still enclosed in membrane when they are shed from the cell and unless this breaks down the spherules will not affect the chemical composition of the urine, a possible advantage if urine is stored for some time before release.

The relative proportions of the materials in the spherules vary in the different genera. Delhaye (1976) noted that melanins are more abundant and ferric iron less in the spherules of *Patella* than in *Monodonta* and *Diodora* (similar to *Emarginula*). This is reflected in the ultrastructural appearance of the spherules described below and shown in plates 2, 3, 5 and 6.

The mechanism of formation is similar in the three genera, however, insofar as can be deduced from electron micrographs. Delhaye demonstrated pinocytosis as the process by which the excretory cells of *Monodonta* abstracted ferritin injected into the blood; this observation is confirmed by the identification of coated vesicles at some stages of the cells' activity, both on the basal membrane and in the underlying cytoplasm. Vesicles are seen in the process of fusing with excretory vacuoles of varying size and may appear as evaginations of the vacuolar membrane (plate 2, figure 9, arrowed) or sometimes bulge into the vacuole as if being nipped off from the cytoplasm (also arrowed). Large vacuoles in the apical cytoplasm are ultimately shed in blebs of cytoplasm nipped off from the cell membrane. The reported rupture of vacuolar membranes throughout the cell (Andrews 1981) is now believed to have been due to deterioration in snails which suffered in transit from distant collecting sites, since it has not been found when fixation was carried out soon after collecting.

The basal surface area of the cells is greatly enhanced by a tightly interwoven network of primary and secondary branches forming a distinct zone (figure 8) the tips of the latter being attached to the basal lamina by hemidesmosomes. This has sometimes been likened to the development of pedicels at a filtration site but there are no diaphragms, there is limited contact between branches and basal lamina, and the volume of extracellular space is small relative to that at a filtration site (cf. plate 1). The branches have a supporting framework of microfibrils. Mitochondria tend to be more abundant in the basal regions of the cells than elsewhere, Golgi

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Description of plate 1

Figures 4–7. The epicardium of the auricle in *Emarginula*.

Figure 4. Transverse section of the auricle showing podocytes over the filtration site. Scale bar, 2 μm.

Figure 5. Detail of raised pedicels and deposits of glycogen. Scale bar, 1 μm.

Figure 6. Detail of the intercellular junction at the pericardial end of the intercellular space. Scale bar, 0.5 μm.

Figure 7. Pedicels overlying the basal lamina. Scale bar, 250 nm. Inset: surface view showing filtration slit bridged by a diaphragm. Scale bar, 250 nm. am, Amoebocyte; bl, basal lamina; cf, collagen fibres; cp, coated pit; d, diaphragm, surface view indicated by arrows; fs, filtration slit; gly, glycogen; ics, intercellular space; j, intercellular junction; lau, lumen of auricle; mf, muscle fibre; mi, mitochondrion; n, nucleus; pc, pericardial cavity; pe, pedicels; po, podocyte; rpe, raised pedicels; scs, subcellular space; v, vesicle.

Description of plate 2

Figures 8 and 9. The right kidney of *Monodonta*, proximal region.

Figure 8. Part of the cuboidal epithelium. Scale bar, 10 μm.

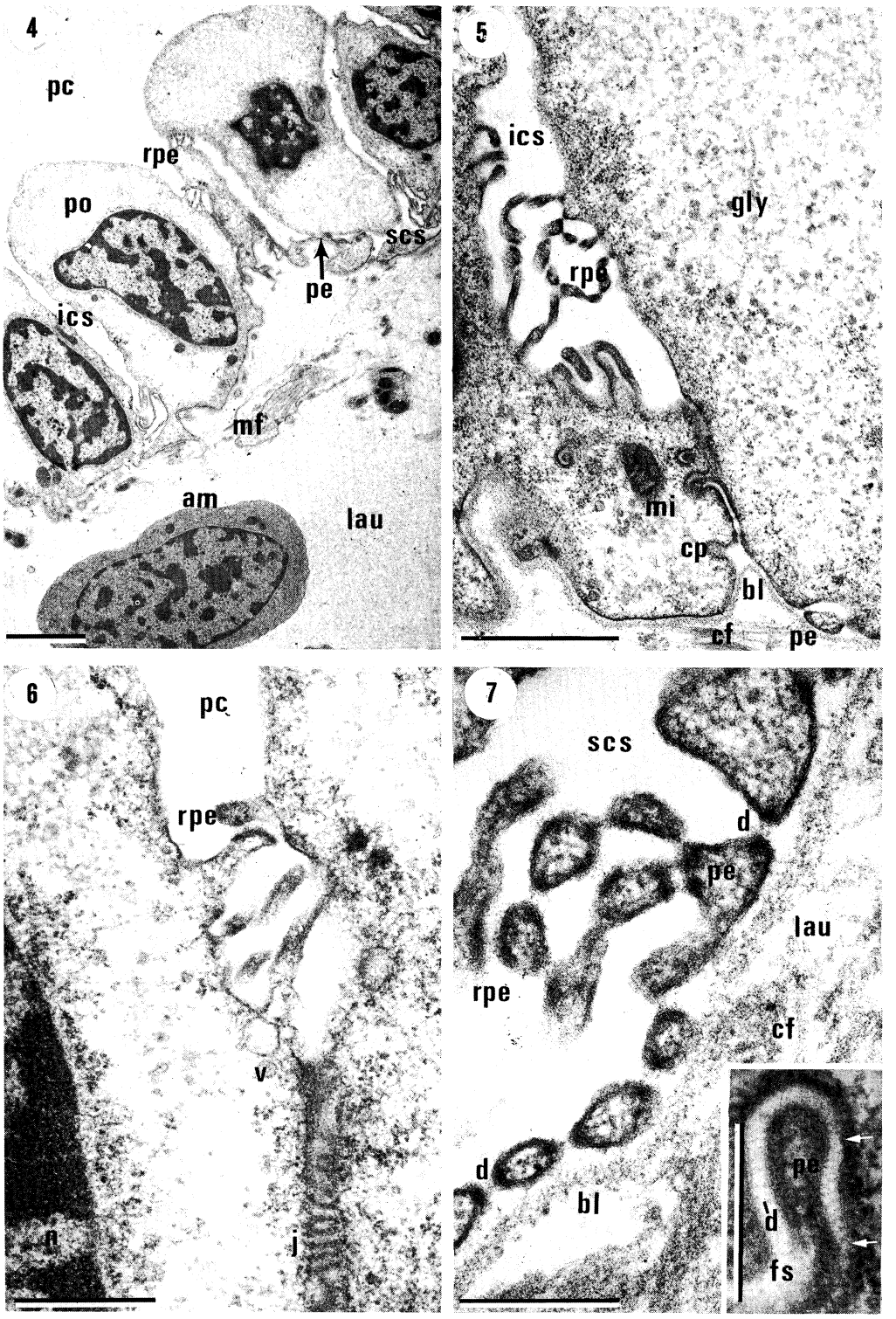
Figure 9. The apical regions of excretory cells during a phase when blebs of cytoplasm are being nipped off. Scale bar, 2 μm. ble, Blebs of cytoplasm; bpr, basal processes; bs, blood space; ci, cilia; egr, excretory granule; ev, excretory vacuole; lc, lipofuscin core; lk, lumen of kidney; ly, lysosome; mf, muscle fibre; mi, mitochondrion; mv, microvilli; n, nucleus; nf, nerve fibre; ply, primary lysosome.

Description of plate 3

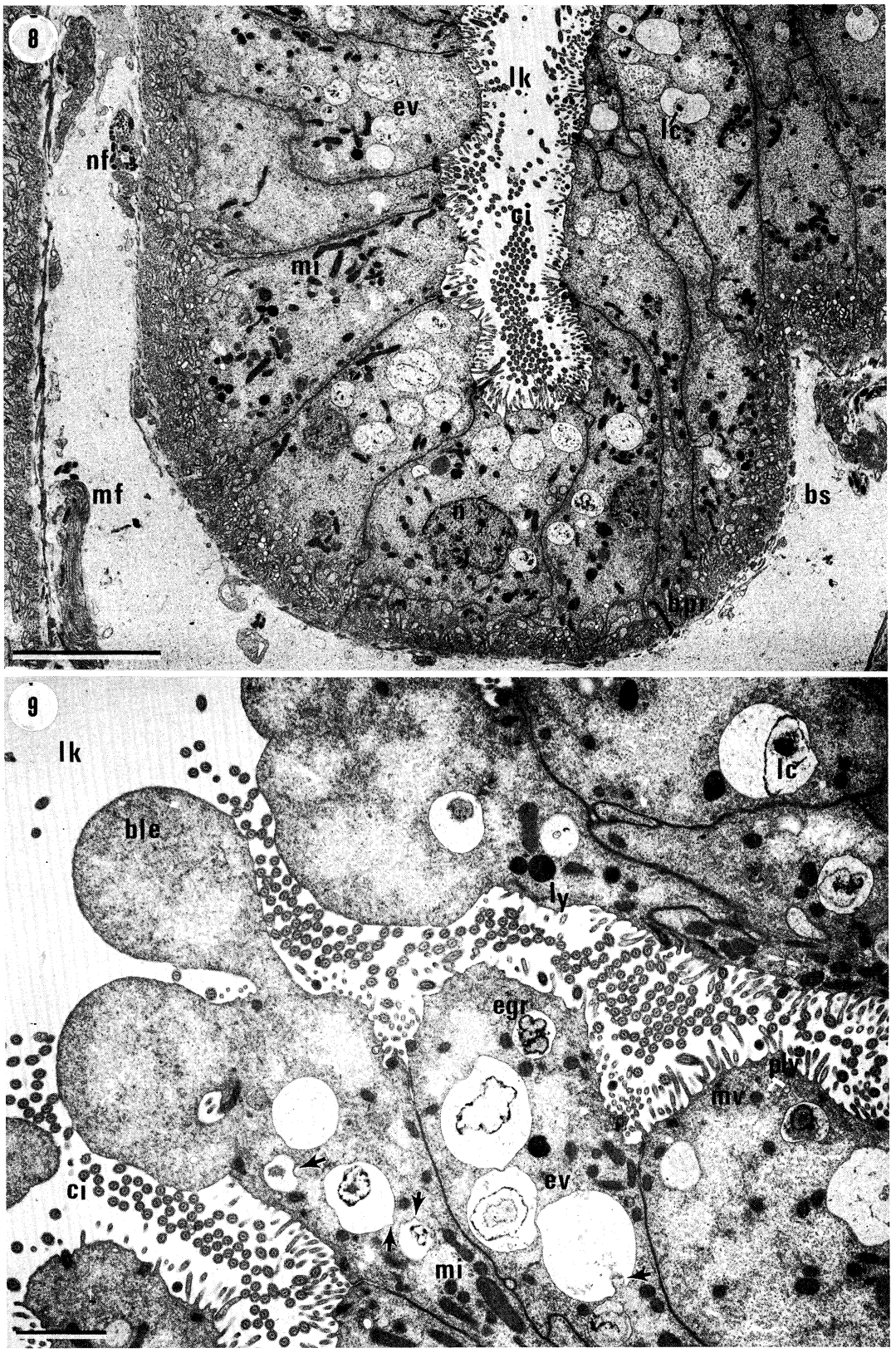
Figures 10 and 11. The right kidney of *Monodonta* distal region.

Figure 10. Part of the columnar epithelium. Scale bar, 2 μm.

Figure 11. Detail of the basal region of the epithelium. Scale bar, 1 μm. bl, Basal lamina; bpr, basal processes; egr, excretory granule; ev, excretory vacuole; gb, golgi body; if, infolding of basal membrane; ly, lysosome; mi, mitochondrion; mv, microvilli; n, nucleus.



FIGURES 4–7. For description see opposite.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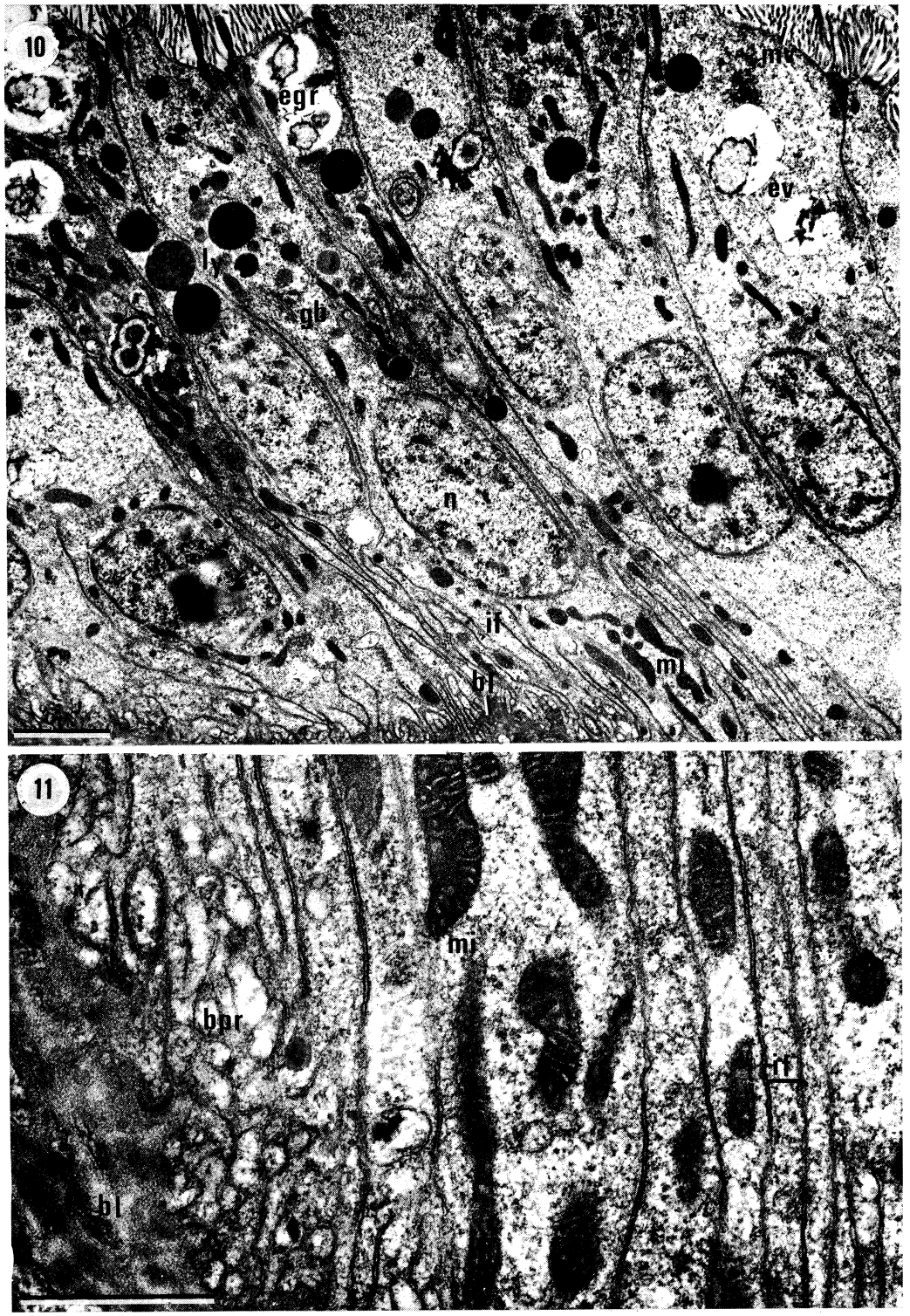
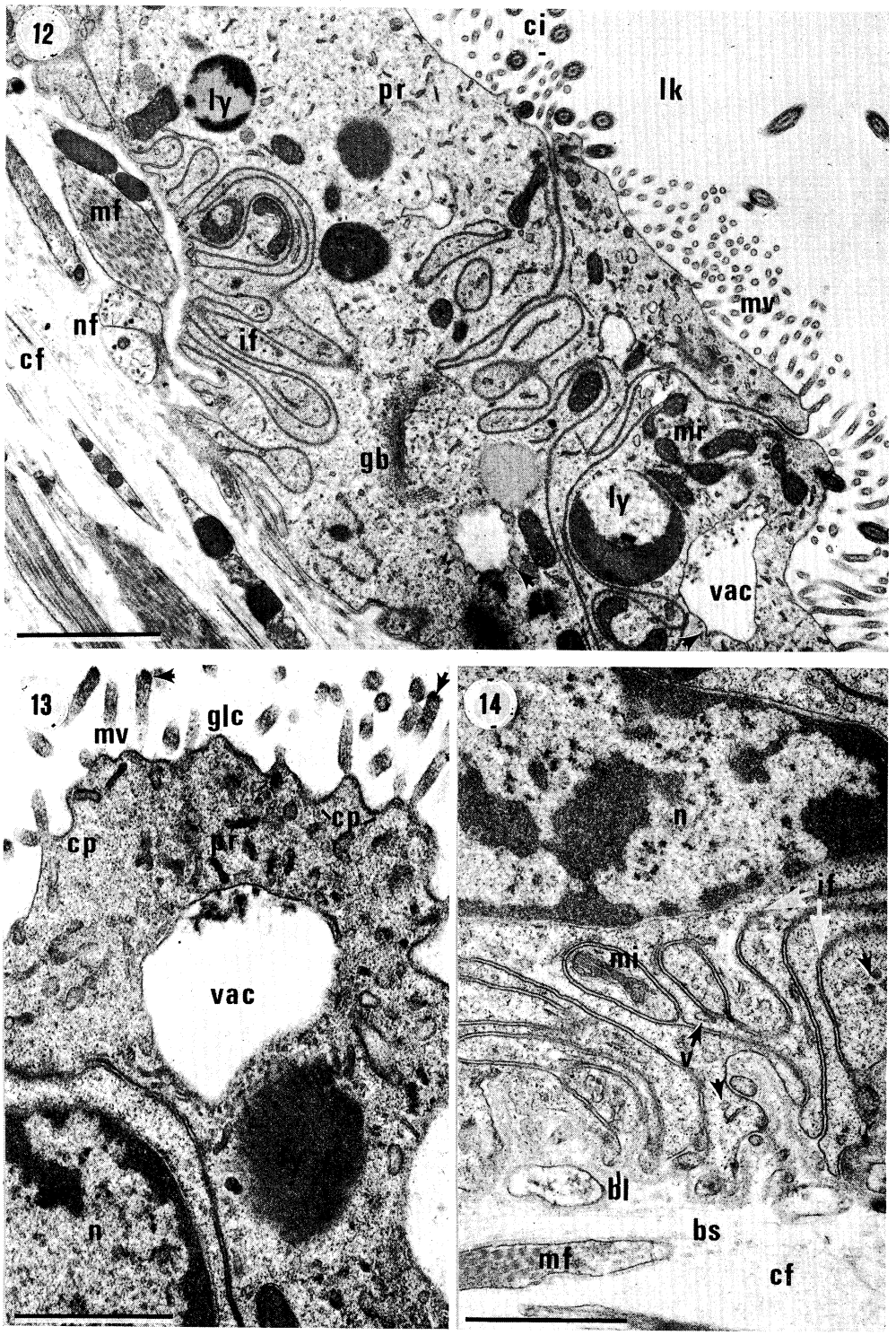


FIGURES 10 AND 11. For description see p. 394.



FIGURES 12–14. For description see opposite.

Description of plate 4

Figures 12–14. The right kidney of *Emarginula*, distal region.

Figure 12. The squamous-cuboidal epithelium. Scale bar, 2 μm.

Figure 13. Detail of the apical cytoplasm to show pinocytotic activity. Scale bar, 1 μm.

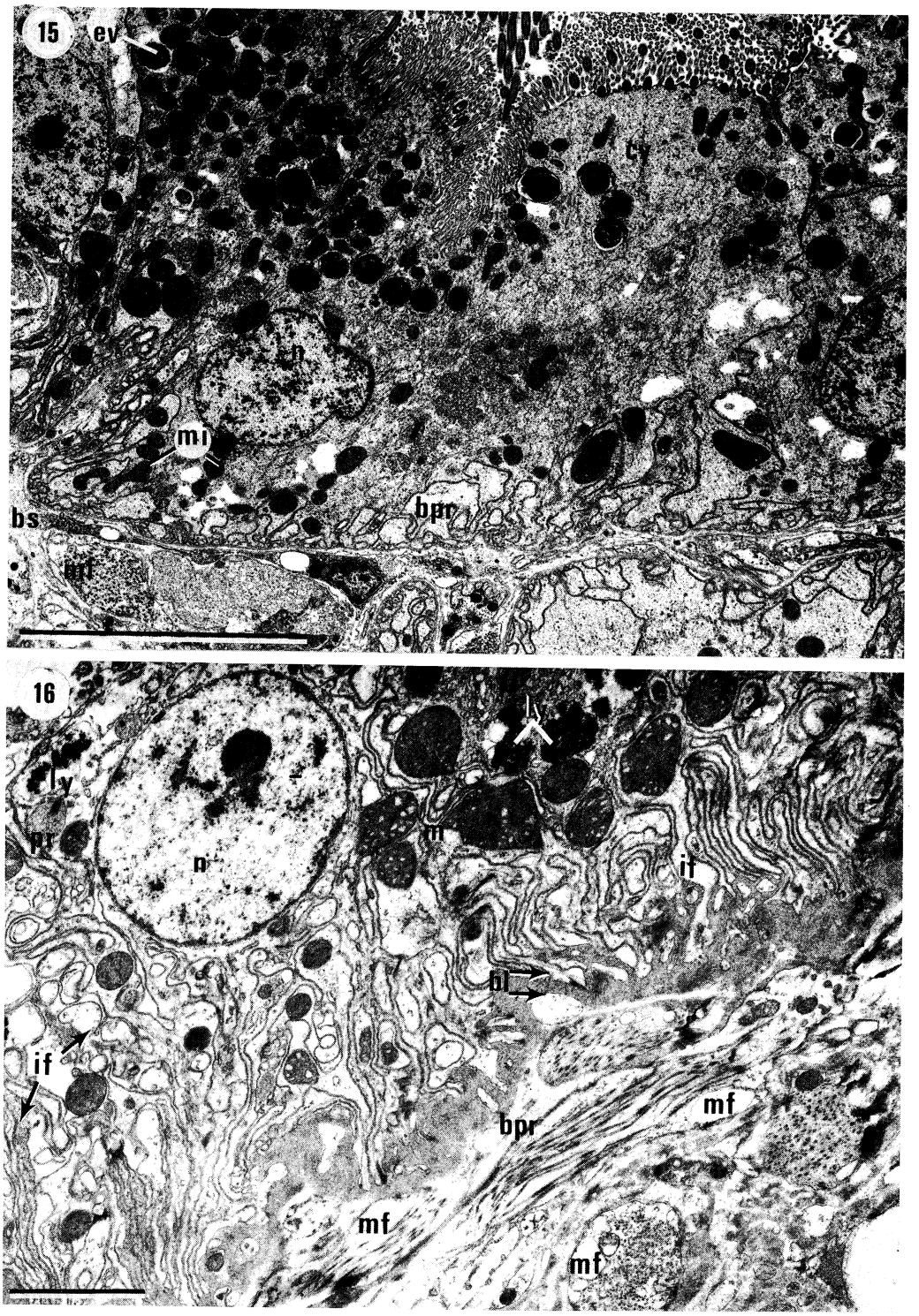
Figure 14. Detail of the basal region of an epithelial cell to show basal infoldings. Scale bar, 1 μm. bl, Basal lamina; bs, blood space; cf, collagen fibres; ci, cilia; cp, coated pits; gb, golgi body; glc, glycocalyx; if, infolding of basal membrane; lk, lumen of kidney; ly, lysosome; mf, muscle fibre; mi, mitochondrion; mv, microvilli (electron dense tips arrowed); n, nucleus; nf, nerve fibre; pr, profiles of pits; v, pinocytotic vesicles (arrowed); vac, vacuole into which contents of coated pits are shed.

Description of plate 5

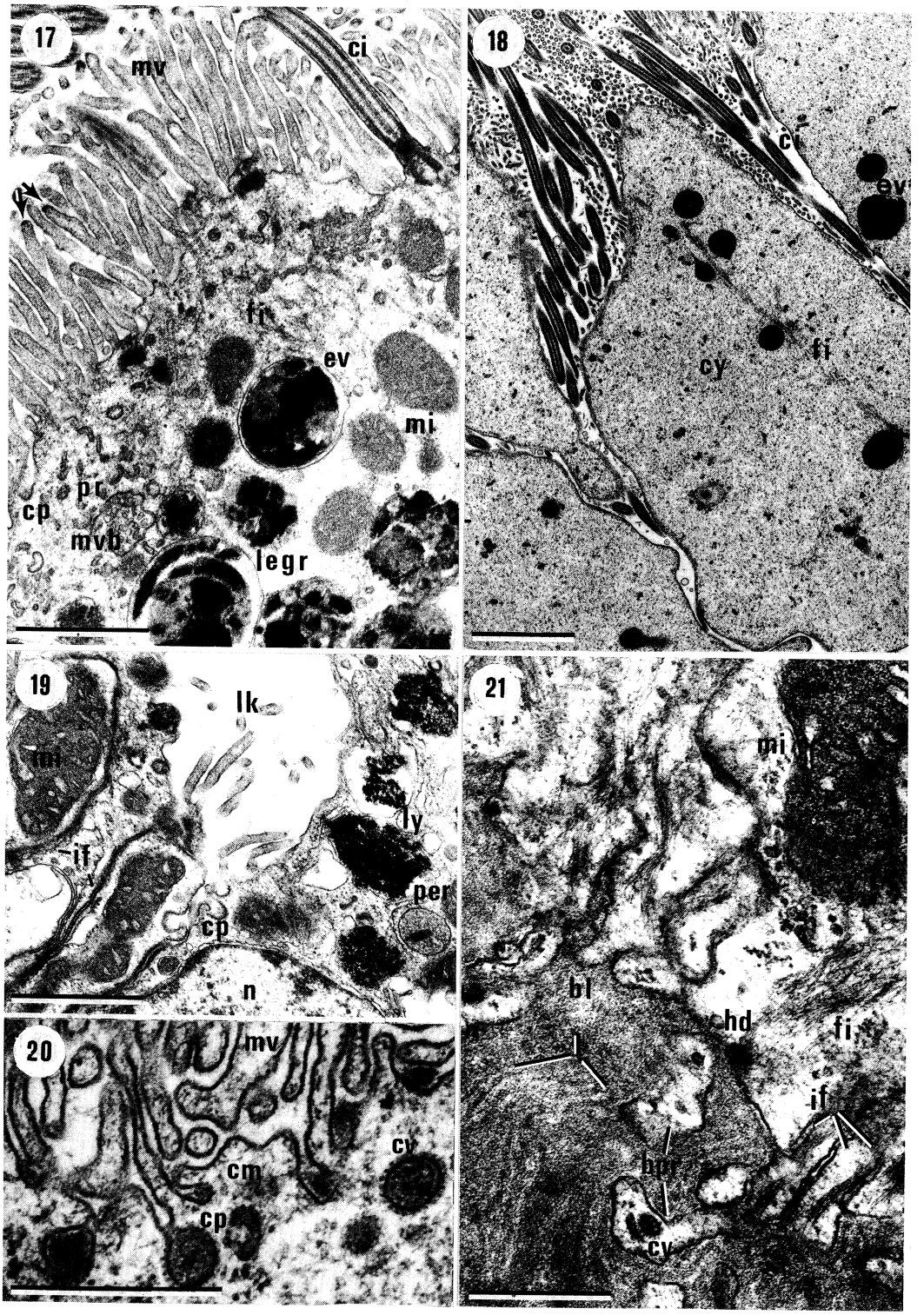
Figures 15 and 16. The right and left kidneys of *Patella*.

Figure 15. Epithelium of the right kidney, proximal region. Scale bar, 10 μm.

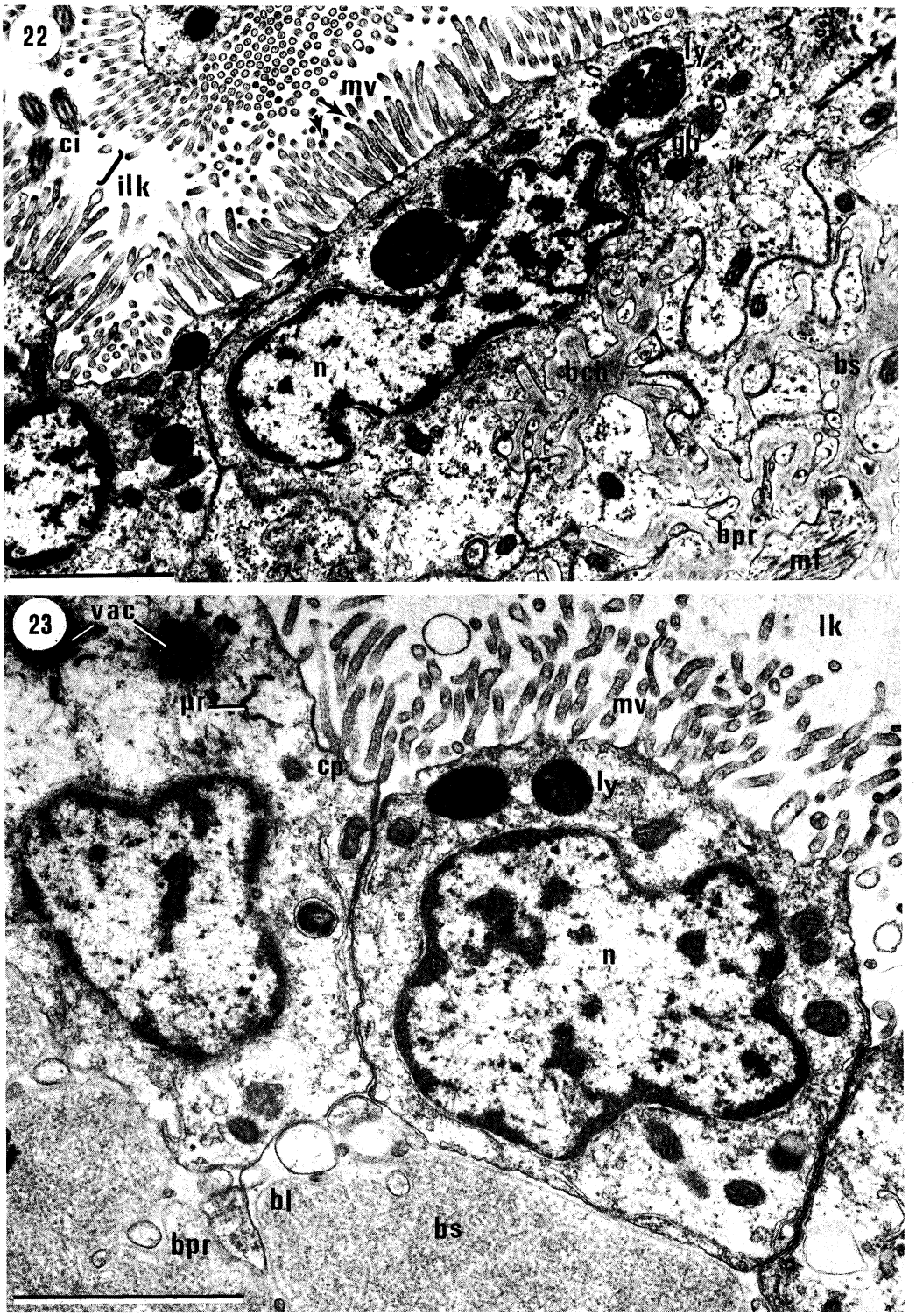
Figure 16. Epithelium of the left kidney. Scale bar, 2 μm. bl, Basal lamina; bpr, basal processes; bs, blood space; cy, ground cytoplasm; ev, excretory vacuole; if, infolding of basal membrane; ly, lysosome; mf, muscle fibre; mi, mitochondrion; n, nucleus; pr, profiles of pits.



FIGURES 15 AND 16. For description see opposite plate 4.



FIGURES 17–21. For description see opposite plate 7.



FIGURES 22 AND 23. For description see opposite.

Description of plate 6

Figures 17–21. Detail of renal epithelium of *Patella*.

Figure 17. Peripheral apical region of an epithelial cell of the right kidney. Scale bar, 1 μm.

Figure 18. Central apical region of an epithelial cell of the right kidney. Scale bar, 2 μm.

Figure 19. Apical region of an epithelial cell of the left kidney. Scale bar, 1 μm.

Figure 20. Detail of apical cell membrane and coated pits. Scale bar, 0.5 μm.

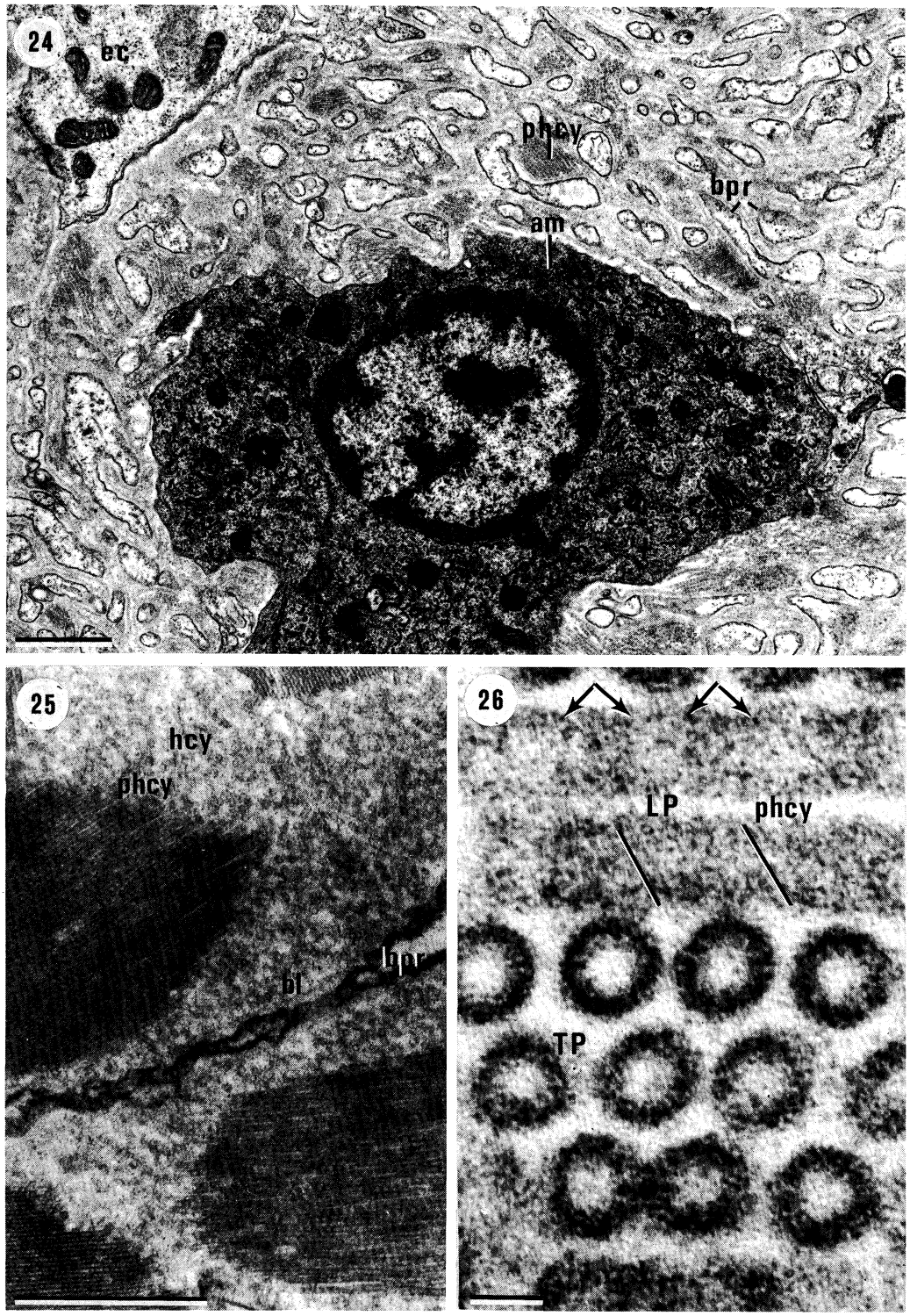
Figure 21. Basal region of an epithelial cell of the left kidney and the basal lamina. Scale bar, 0.5 μm. bl, Basal lamina; bpr, basal processes; ci, cilia; cm, coated inner cell membrane; cp, coated pit; cv, coated vesicle; cy, ground cytoplasm; ev, excretory vacuole; fi, fibril; hd, hemidesmosome; if, infolding of basal membrane; legr, concentric layers of excretory granule; lk, lumen of kidney; ly, lysosome; mi, mitochondrion; mv, microvilli (dense tips arrowed); mvb, multivesicular body; n, nucleus; per, peroxisome; pr, profiles of pits.

Description of plate 7

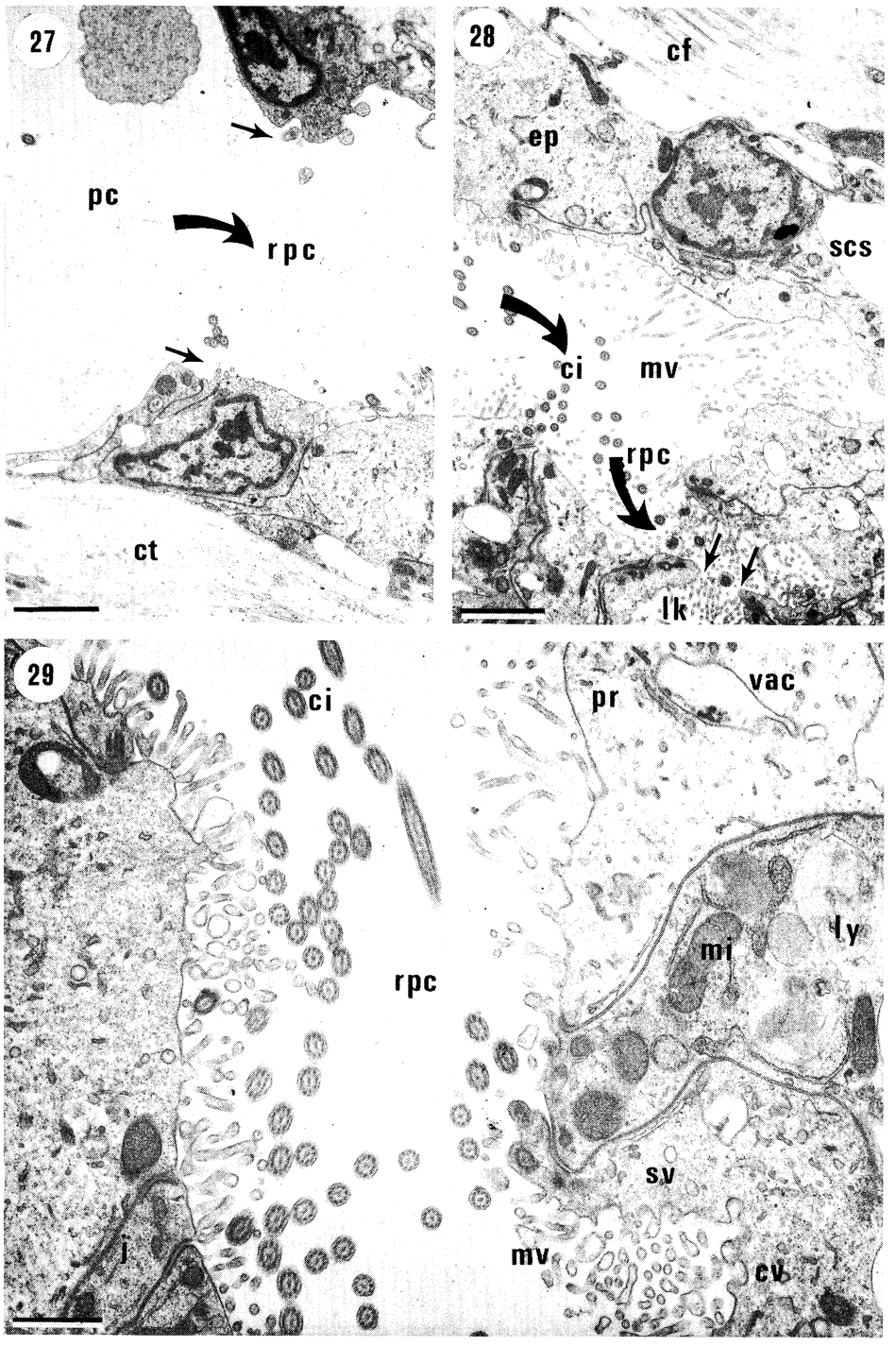
Figures 22 and 23. Epithelium of the left kidney of *Monodonta*.

Figure 22. Epithelium lining the tubular invaginations of the nephridial gland. Scale bar, 2 μm.

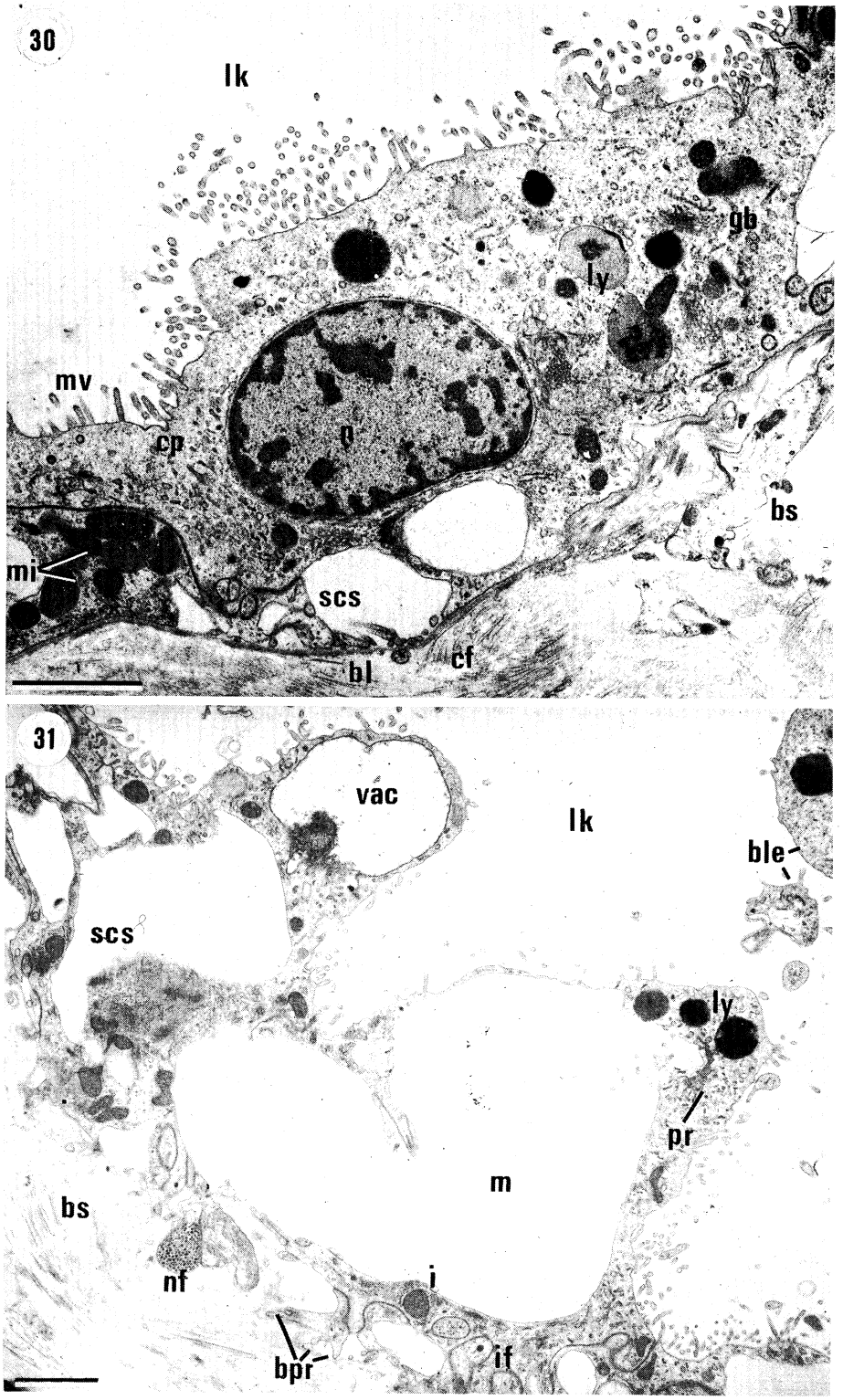
Figure 23. Epithelium over the surface of the papillae. Scale bar, 2 μm. bch, Blood channels invaginating cell body; bl, basal lamina; bpr, basal processes; bs, blood space; ci, cilia; cp, coated pits; gb, golgi body; ilk, lumen of kidney invaginating nephridial gland; lk, lumen of kidney; ly, lysosome; mf, muscle fibre; mv, microvilli; n, nucleus; pr, profiles of pits; vac, vacuole into which contents of pits are shed.



FIGURES 24–26. For description see p. 395.



FIGURES 27–29. For description see p. 395.



FIGURES 30 AND 31. For description see opposite.

bodies are usually prominent close to the central nuclei and secondary lysosomes, if found, are in the apical cytoplasm.

The apical cell membrane also bears ultrastructural features associated with transport: the array of microvilli provides a large surface area for diffusion and coated pits are in evidence, opening between microvilli. It seems unlikely that they have traversed the cell, transporting solutes direct from the basal membrane, since coated vesicles have not been found throughout the cytoplasm, so it is probable that they are involved in absorption of solutes from the urine.

The above description applies to excretory cells in all three genera studied, and in *Monodonta* this type of cell lines the main (proximal) part of the kidney (figure 8). In the tubular distal portion, however, there is a transition to taller narrower cells with ovoid nuclei which occupy almost the whole breadth of a cell (figure 10). The basal branches (figure 11, bpr) occupy a smaller proportion of their total height, and the cells have infoldings of basal membrane aligned with dense elongated mitochondria in the cytoplasm above the branches. Harrison (1962) observed some limited glucose uptake in the right kidney of *Haliotis* and it is possible that some active transport may occur in this part of the kidney in *Monodonta*.

The fine structure of the excretory cells of *Emarginula* has been briefly described (Andrews 1981). The vacuoles vary considerably in appearance and are difficult to preserve undamaged

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Description of plate 8

Figures 24–26. The blood spaces of the left kidney of *Monodonta*.

Figure 24. Blood space of the nephridial gland invaded by numerous processes of epithelial cells and amoebocytes. Scale bar, 1 μm.

Figure 25. Blood space of a papilla containing pseudocrystalline aggregations of haemocyanin. Scale bar, 0.5 μm.

Figure 26. Detail of polymerized haemocyanin. Scale bar, 20 nm. am, Amoebocyte; bl, basal lamina; bpr, basal process; ec, epithelial cell; hcy, haemocyanin; LP, chains in longitudinal plane; phcy, polymerized haemocyanin; TP, chains in transverse plane. Arrows indicate periodicity along chain of molecules; parallel lines delineate section which appears to show a spiral pattern.

Description of plate 9

Figures 27–29. The left renopericardial canal of *Emarginula*.

Figure 27. Opening of the renopericardial canal into the pericardial cavity. Scale bar, 2 μm.

Figure 28. Opening of the renopericardial canal into the lumen of the kidney. Scale bar, 2 μm.

Figure 29. Apical regions of epithelial cells lining the canal. Scale bar, 1 μm. cf, Collagen fibres; ci, cilia; ct, connective tissue; cv, coated vesicle; ep, epithelium; j, intercellular junction; lk, lumen of kidney; ly, lysosome; mi, mitochondrion; mv, microvilli; pc, pericardial cavity; pr, profiles of pits; rpc, renopericardial canal; scs, subcellular space; sv, smooth vesicle; vac, vacuole into which contents of pits are shed. Small arrows indicate margins of renopericardial openings, large arrows indicate presumed direction of ciliary currents.

Description of plate 10

Figures 30 and 31. The left kidney of *Emarginula*.

Figure 30. The epithelium of the left kidney in transverse section. Scale bar, 2 μm.

Figure 31. The epithelium cut obliquely in a phase when blebs of apical cytoplasm are nipped off. Scale bar, 2 μm. bl, Basal lamina; ble, blebs of cytoplasm free in lumen; bpr, basal processes; bs, blood space; cf, collagen fibres; cp, coated pits; gb, golgi body; if, infolding of basal membrane; lk, lumen of kidney; ly, lysosome; mi, mitochondria; mv, microvilli; n, nucleus; nf, nerve fibre; pr, profiles of pits; scs, subcellular space; vac, vacuole into which pits shed their contents.

during specimen preparation. However, in almost all respects they show a striking resemblance to the excretory cells of *Monodonta*.

The smooth-walled part of the kidney near its external opening may be homologous with the tubular portion in *Monodonta* and like this shows features associated with active transport such as basal infoldings and mitochondria, though the epithelium here is low, in contrast to that in *Monodonta* (figure 12). The cells in this region have a somewhat irregular array of widely spaced microvilli punctuated by numerous pits, in some of which the inner membranes are coated. Lysosomes (ly), irregular vacuoles (vac) and pits in various planes of section (pr) are the major features of the apical cytoplasm (figure 13) together with long narrow mitochondria (mi) lying parallel to the apical membrane. All these suggest endocytosis at specific receptor sites, followed by lysosomal fusion. The cells have basal infoldings (figure 14) and while there are some mitochondria in adjacent cytoplasm, they do not present the regular arrangement usually associated with transporting epithelia.

While there are strong resemblances in the ultrastructure of the excretory cells in *Monodonta* and *Emarginula* the similarity is less obvious in *Patella*, in which all the cells are strongly ciliated and have a dense border of tall microvilli with electron-dense tips (figure 15). The coated pits of the membrane and vesicles in the underlying cytoplasm are more conspicuous than in excretory cells of other genera. The presence of multivesicular bodies (figure 17, mvb) in the cytoplasm suggests that the vesicles may fuse with them, one of the pathways of endocytosis discussed by Geisow (1980). Many dense ovoid mitochondria (mi) occur in the apical cytoplasm, interspersed with excretory vacuoles (ev). The excretory masses are much more heterogeneous than those of the other genera and do not have a crystalline appearance, due to the large quantity of melanin they contain. Peroxisomes (per) occur in the basal cytoplasm, but in *Monodonta* and *Emarginula* these have been found only in the left kidney. There is a clear grouping of cells in particular phases the most conspicuous of which is one in which the apical cytoplasm loses its organelles and is reduced to a finely granular mass containing excretory vacuoles (figure 18, cy). At this stage the cells bulge into the lumen and ultimately lose their microvilli over this area. The masses nipped off lie in the lumen together with the remains of whole cells that have degenerated.

There are extensive subepithelial blood spaces in the right kidney of all three genera and they contain occasional amoebocytes. In *Monodonta* and *Emarginula* there are moderate concentrations of haemocyanin in the spaces, and there are never large aggregates of the pigment, as there are in the left kidney (plate 8). In *Patella* however, there is no trace of haemocyanin; instead there appears to be a different type of blood protein of smaller molecular size, also seen in electron micrographs of *Lepidochitona* (Andrews 1986) but not in other gastropods so far examined. It may be significant that haemocyanin appears to be absent in *Lepidochitona* too.

The left kidney

The most conspicuous difference in the ultrastructure of the left kidney by comparison with the right is the lack of excretory vacuoles and an abundance of secondary lysosomes. This is correlated with far greater numbers of coated pits, vesicles and profiles of pits (earlier believed to be short lengths of smooth endoplasmic reticulum) in the apical cytoplasm (plates 6 and 9), suggesting a much greater resorptive capacity than that of the right kidney, as seen in Harrison's work on *Haliotis* (1962).

Transport across the epithelium occurs in the opposite direction too, from blood to lumen, as demonstrated by Delhaye (1976) when he injected ferritin into the blood of *Monodonta*. A marked increase in vesicles in the basal cytoplasm of the epithelium was accompanied by accumulation of ferritin in multivesicular bodies, lysosomes and residual bodies. These organelles are conspicuous in the epithelium of the left kidney in the three genera investigated here and seen ultimately to be expelled by apocrine secretion.

Transepithelial transport is enhanced by the large basal surface area offered by long branches the pattern of which varies from one genus to another but never takes the form of the complex and compact network characteristic of the right kidney.

In *Monodonta* the epithelium of the left kidney is composed of two types of cell not clearly differentiated in an earlier description (Andrews 1981), one type confined to the nephridial gland (figure 22). The blood-filled papillae that typify this organ are covered by cuboidal epithelial cells (figure 23) sending long thin basal branches (bpr) deep into the central blood space, from which they are separated by only a thin basal lamina. The apical cytoplasm shows great activity, with numerous lysosomes and indications of coated vesicles derived from the apical membrane fusing with them (vac), suggestive of selective uptake of solutes. Experimental work in progress also indicates a high rate of uptake of radioactive glucose in the papillary sac of *Monodonta* by a mechanism which is ouabain-sensitive, and unlikely to involve transport in vesicles. This suggests therefore that there are at least two different pathways for transport of solutes across these cells.

The epithelium in the nephridial gland (figure 22) is distinguished by a network of narrow blood channels (bch) permeating the cell bodies to such an extent that in places they almost touch the cell membrane, a feature described in an earlier account (Andrews 1981) when its restriction to the nephridial gland was not specified. There are also infoldings of the basal membranes associated with mitochondria, suggesting involvement of the gland in ion transport. In the nephridial gland, the subepithelial connective tissue is much richer in cells than the papillae, and the blood spaces narrower (figure 24). While the concentration of haemocyanin is high in both the large pseudocrystalline aggregations characteristic of the papillae (figures 25 and 26) do not occur in the gland.

In *Emarginula* the walls of the minute left kidney and its renopericardial canal bear a low epithelium, which at the ultrastructural level belies its inactive appearance in paraffin sections (figure 30). It certainly appears to be involved in resorption since the cells show the same features in the apical cytoplasm and cell membrane exhibited by those of the papillary sac in *Monodonta* (plate 9). Most cells bear few cilia, but those near the opening and lining the renopericardial canal are strongly ciliated. Intense pinocytotic activity is apparent apically, coated vesicles and lysosomes being prominent in the cytoplasm. There is a dense covering of microvilli on the apical membrane. Vacuoles of irregular shape and profiles of pits with finely granular contents are prominent in the cytoplasm. Blebs of cytoplasm are seen being nipped off and lying free in the lumen of the kidney at certain phases of activity (figure 31, ble).

In well fixed animals subcellular spaces are extensive, though fairly shallow (plate 10), and in places the basal lamina is exposed. The arrangement is different from that in the filtration site of the auricle, however: there are no pedicels with diaphragms, and there is no indication that these subcellular spaces open to the lumen of the kidney (cf. plates 1 and 10). Many minute vesicles occur in the basal cytoplasm of the cells but there is no regular infolding of the basal membrane usually associated with ion-transporting cells.

The basal lamina is thinner than that of the right kidney and the underlying vascular connective tissue contains collagen (cf) and muscle fibres, particularly well developed along the renopericardial canal and around the kidney opening. Basal branches of the epithelial cells do not invade the blood space, nor do blood-filled channels permeate the cell bodies, as they do in *Monodonta*.

The left kidney of *Patella* is markedly different from that of *Monodonta* and *Emarginula* in a number of respects. Its epithelial cells are columnar (figure 16) and rest on an unusually thick and complex basal lamina (figure 21, bl) composed of alternating dense and loose-textured layers, the latter perhaps carrying blood close to the basal cell membranes. The underlying connective tissue contains many muscle fibres (mf) and the blood spaces, so extensive in the other genera, are no more than narrow channels in which a relatively rapid circulation might be maintained by muscular contraction. Another striking difference is the absence of haemocyanin from this region, where it is so abundant in *Monodonta* and *Emarginula*.

The cells show some features of transporting cells in the deep infolding of the basal cell membrane, sometimes extending the full height of the epithelium, associated with many mitochondria with conspicuous cristae (figure 19, if). The apical margins of the cells become very irregular when blebs of cytoplasm containing secondary lysosomes are nipped off. During this phase of activity the lumen in the tubular part of the kidney is almost filled by the fragments and by the irregular apical regions of the cells (Andrews 1981). Groups of disintegrating cells have been found occasionally, which appear to be replaced by low cells adjacent to them. It is not known whether or not the cells undergo successive cycles of activity, though the infrequency of senescent cells suggests that they do.

The long basal branches of the epithelium in the papillary sac of *Monodonta* appear to be represented in a vestigial form in *Patella* by very thin, short branches which extend across the basal lamina but pass no deeper (figure 21, bpr). Despite some fundamental differences, both the epithelium itself and the underlying connective tissue throughout the kidney are therefore reminiscent of the nephridial gland of *Monodonta* in presenting features associated with active transport. The thickened tubular region that constitutes almost the whole kidney in *Patella* is clearly the same region that is a nephridial gland in *Monodonta* and in this sense may be taken to be homologous with it. The strong possibility remains that the specialized features associated with resorption, not identified in any pleurotomariacean, have arisen independently in patellacean and trochacean stocks in a region which in the kidney of early gastropods had preadaptations for the role of active transport.

Renal evolution and differentiation in prosobranchs

The marked differences described above in the cellular and anatomical organization of the two kidneys of *Emarginula* and *Patella*, already recognized in *Monodonta* and pleurotomariaceans, confirm a consistent pattern of organization in the excretory system of archaeogastropods. This contrasts with the situation presented by previous accounts in which fissurellaceans and patellaceans were purported to have right and left kidneys similar at the histological level. It follows that the two kidneys are likely to have had different functions in the earliest gastropods and that this departure from the typical molluscan plan must in some way be a consequence of gastropod organization. The explanation offered here rests on the proposition that it is closely linked to helical coiling.

It may be supposed that in a bilaterally symmetrical pregastropod (figure 32*a*) the kidneys were similar in structure and function as in other molluscs. In an archaeogastropod with helical coiling the effects of a relative increase in the growth rate on the post-torsional left side of the visceral hump should logically include an increase in size of the left kidney relative to the right if the organs remain in their original position. In reality the converse is true, the right becoming the more extensive, even in pleurotomariaceans (figure 32*b*) and trochaceans in which the left kidney is a large papillary sac (ps) (Fretter 1964, 1966).

One of the first accompaniments of coiling seems to have been the displacement of the left kidney from its primitive position lateral to the pericardium at the base of the visceral hump to one anterior to it, involving some invasion of the mantle skirt as in *Perotrochus* (Fretter 1966). The mantle cavity has deepened in pleurotomariaceans but the ctenidia do not extend into the deepest recesses (figure 32*b*, pbr) and so a space is created in the posterior part of the mantle cavity, especially on the left, into which the kidney, limited in other directions by pericardium and stomach, can spread. In its new situation it is almost completely isolated from the viscera and it is bathed on the pallial side by water in the mantle cavity, not by blood or tissue fluid. It is therefore subject to conditions different from those surrounding the right kidney. The permeable epithelia of the ctenidia and mantle skirt are sites of gaseous and probably ion exchange and there may also be uptake of organic solutes, so altering the composition of the blood in this part of the body.

The right kidney (rk) by contrast is displaced for the most part to a position posterior to the pericardium, having expanded to form a large body cavity by invading spaces between the coils of intestine and lobes of the digestive gland with which it has extensive contact: again exemplified by *Perotrochus*. Since it is this kidney that has retained the primitive association with the viscera, and in particular with the digestive gland, which both kidneys must have had in a pregastropod, it is not surprising that it has retained its traditional role in excretion. This organization of the two kidneys exists in pleurotomariaceans and trochaceans, and the functional implications may best be considered in relation to these groups before discussing the possible origins of the more specialized condition in fissurellaceans and patellaceans.

One of the most profound consequences of this rearrangement of the kidneys has been the disruption of the original blood supply to the left kidney and the acquisition of a new one. It may be assumed that in a pregastropod blood from the visceral and cephalopedal haemocoels would be apportioned equally to the kidneys by paired afferent renal veins, as it is in other molluscs. Right and left efferent renal veins then carried blood from the renal sinuses to the mantle skirt and ctenidia (figure 32*a*). Such an arrangement does not exist in any living archaeogastropod: while the right kidney conforms to this plan, the left does not. In *Haliotis* nearly all blood passes through the right kidney on its way to the basibranchial sinus, supplying the mantle skirt and respiratory organs (Crofts 1929). In the pleurotomariacean *Mikadotrochus* (Fretter 1964) and in trochids (Nisbet 1953) blood from the visceral hump, which probably has the highest concentration of waste metabolites, takes this route, while some or all from the head-foot goes direct to the mantle skirt.

The left kidney too retains a connection with the basibranchial sinus of pleurotomariaceans or its homologue, the transverse pallial vein, in trochaceans. There has been some debate as to the direction of blood flow in this vessel (figure 32*b*, lerv), summarized by Fretter & Graham (1962), since the left kidney has lost its afferent renal vein and the new source of its blood has been in doubt. However, both Crofts (1929) and Fretter (1966) have adopted the view that

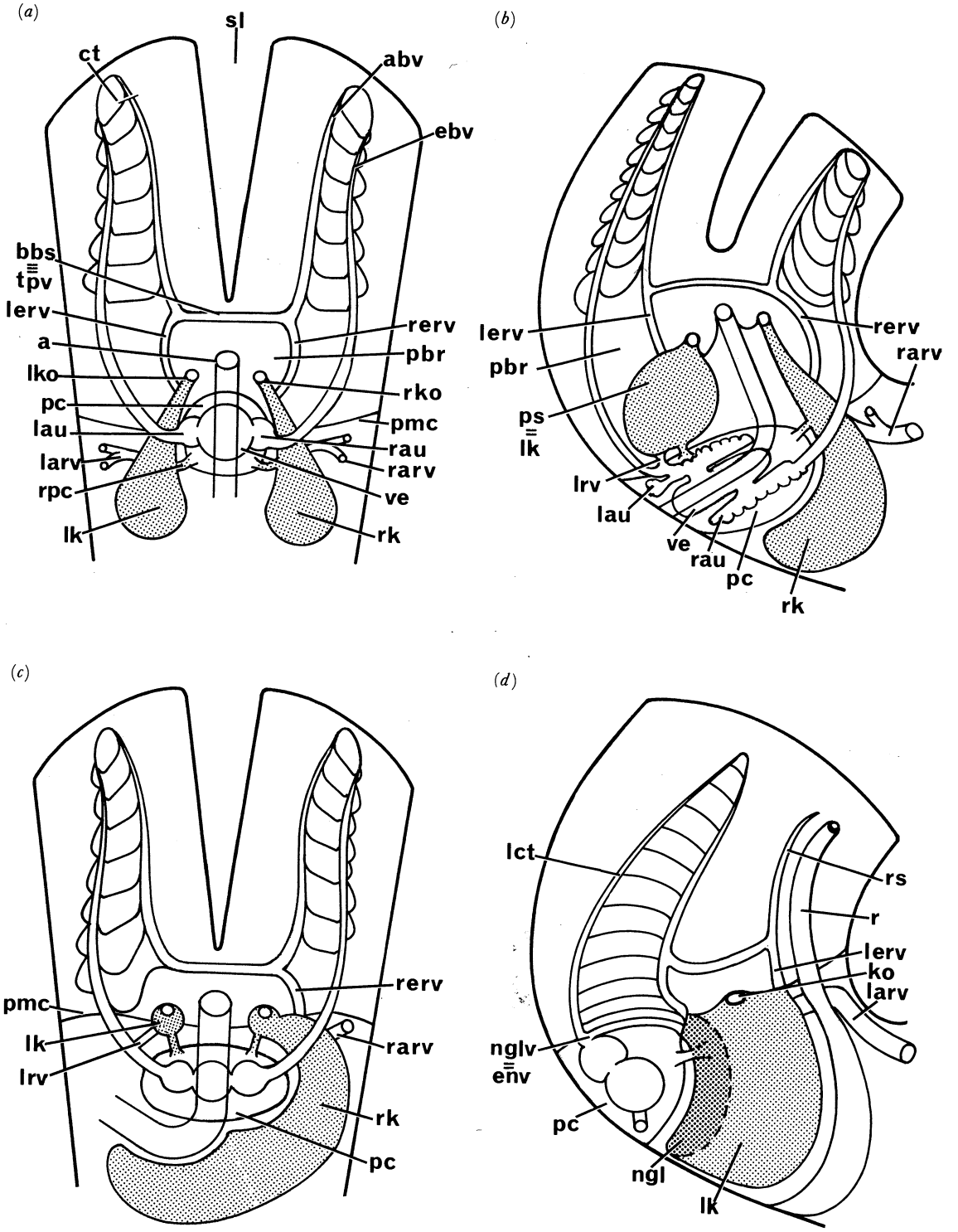

FIGURE 32. Diagrammatic representation of the heart, kidneys and related structures in (*a*) a hypothetical primitive gastropod; (*b*) a pleurotomariacean; (*c*) a fissurellacean; (*d*) a monotocardian. a, Anus; abv, afferent branchial vein; bbs, basibranchial sinus; ct, ctenidium; ebv, efferent branchial vein; env, efferent vein of nephridial gland; ko, kidney opening; larv, left afferent renal vein; lau, left auricle; lct, left ctenidium; lerv, left efferent renal vein; lk, left kidney; lko, left kidney opening; lrv, left renal vein; ngl, nephridial gland; nglv, nephridial gland vein; pbr, postbranchial region of mantle skirt; pc, pericardial cavity; pmc, posterior wall of mantle cavity; ps, papillary sac; r, rectum; rarv, right afferent renal vein; rau, right auricle; rerv, right efferent renal vein; rk, right kidney; rko, opening of right kidney; rpc, renopericardial canal; rs, rectal sinus; sl, slit in mantle skirt; tpv, transverse pallial vein; ve, ventricle.

the vessel to the mantle skirt still serves as an efferent route in the pleurotomariaceans they studied, while a connection between the left efferent ctenidial vein and kidney is the afferent (lrv). This connection is apparently a gastropod innovation, replacing the lost left afferent renal vein. There is evidence to suggest, moreover, that it serves not only as the new afferent but also as the major efferent route of the left kidney, returning blood to the auricle. In *Haliotis* the original left efferent renal vein is reduced. When Crofts injected dye into the left efferent ctenidial vein it always entered the left kidney via the new connection more readily than it did the auricle. Furthermore, on one occasion a bubble accidentally introduced on injection was observed to move from the auricular opening to the kidney and back again for a period of two hours, suggesting that new vessel served as both an afferent and main efferent vein for the left kidney.

The original efferent vein is well developed in *Monodonta* but its functional significance in this animal is made obscure by the additional complication of its connection to the left auricle by the posterior renal vein (figure 1, prv). The valve-like effect of a double bend at its junction with the original efferent vessel suggests that any blood travelling along it from the auricle would only pass beyond the double bend if pressure were to rise above a threshold on the auricular side. At lower pressures it would seem that blood is more likely not to flow beyond the bend but to ebb and flow through the branches of the vein in the kidney with the pulsation of the auricle.

Development of this transverse vessel linking the original efferent vein with the left auricle may be correlated with that of the nephridial gland, since neither is found in a pleurotomariacean. It provides a link with the auricle by which a one-way circuit might operate in the nephridial gland, the vessel along the left margin of the gland (lngv) acting as afferent, that on its right margin (mngv) guarded by a semilunar one-way valve as efferent.

The probability that a high proportion, if not all of the blood supplying the left kidney (and in particular its nephridial gland) is postbranchial in *Monodonta* has considerable physiological implications (figure 33). Blood returning from the left ctenidium to the heart is oxygenated, ammonia is likely to have diffused out of the blood across the epithelia of both right kidney and ctenidium and therefore to occur in low concentration, and other forms of nitrogenous waste will have been abstracted by the right kidney.

Some of these factors would contribute to a higher pH in postbranchial than in prebranchial blood. While there are no measurements to substantiate this claim in archaeogastropods Mangum & Polites (1980) have recorded values of pH 7.85 and 8.04 respectively for pre- and postbranchial blood in the neogastropod *Busycon*.

The primitive vascular arrangements persist in the right kidney: it receives prebranchial blood almost at the end of its circuit round the body when oxygen tension is low, carbon dioxide tension high, and pH probably at its lowest in the body. It will also be laden with the products of metabolism. The right kidney is therefore well placed to undertake nitrogenous excretion.

The marked difference in the concentration and degree of polymerisation of the haemocyanin in the blood of the two kidneys also has important implications. The concentration in the right kidney is relatively low, but in the left blood spaces are typically densely packed, both with single molecules and chains similar to those described by Wood (1980). The chains have been shown to arise *in vitro* at pH 8.00 or above in the presence of Ca^{2+} ions. The normal (9×10^6 Da) haemocyanin molecule is known to be stable between pH 7.6–8.0 but at a higher pH in the presence of divalent cations the molecule dissociates into smaller subunits which can

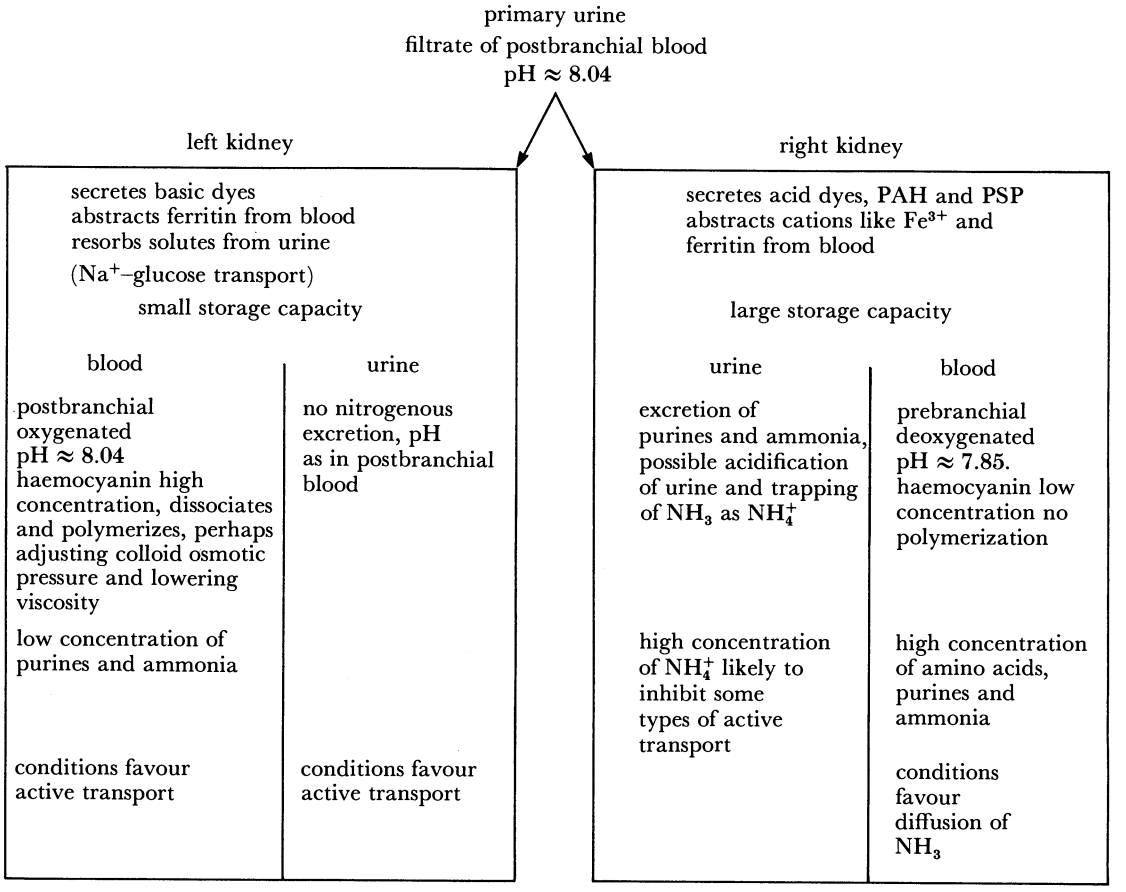

FIGURE 33. A summary of the functions of right and left kidneys of archaeogastropods and the suggested differences in the composition of their blood and urine.

associate to form chains. Wood suggested that this change was unlikely to occur *in vivo* and would seem not to have any respiratory function. Its functional significance may, however, be in connection with resorption not respiration, a high concentration in the left kidney raising the blood osmotic pressure (albeit slightly), and increasing the osmotic gradient across the epithelium favouring water resorption. This in turn could change osmotic gradients in intercellular channels and affect solute transport. This may indicate a preadaptation to conditions in which water and ion uptake are essential, as in intertidal, freshwater and terrestrial prosobranchs. The very existence of the pseudocrystalline arrays in the blood spaces of the left kidney (plate 8) is therefore circumstantial evidence of a pH value of 8.00 or above, suggesting that most if not all the blood in this kidney is postbranchial. Mangum & Polites (1980) not only recorded a pH of 8.04 in the postbranchial blood of *Busycon* but also found a relatively high concentration of divalent cations.

The tendency for haemocyanin to form pseudocrystalline aggregations has also been reported in the nephridial gland of the carnivorous mesogastropod *Lunatia* (= *Natica*) (Fretter & Graham 1962; Andrews 1981) pointing to the possibility that the so-called efferent vein of the nephridial gland of monotocardians may also act as a two-way shunt carrying postbranchial blood, as suggested for its homologue in archaeogastropods.

Chain formation has not been observed in any other part of the blood system, and as oxygen

tension and pH fall the subunits capable of this type of polymerization aggregate into whole molecules which lack this ability. Blood reaching the right kidney of archaeogastropods has a low oxygen tension and is likely to have a pH lower than 7.9, the conditions in which the whole haemocyanin molecule is stable. Even if there were no difference in the concentration of haemocyanin in the blood in right and left kidneys the changes in the state of aggregation of the haemocyanin molecule would result in a lower colloid osmotic pressure in the right kidney than in the left, with attendant differences in the activity of inorganic ions in the blood (as indicated by Mangum & Polites 1980). The osmotic gradient between urine, tissue and blood might therefore be expected to be lower than that in the left kidney in which conditions would appear to be more favourable for resorption. This proposition is consistent with the ultrastructural evidence.

The new relationship between left kidney and blood system also favours regulatory activities involving active transport in that oxygen is readily available in postbranchial blood, but is scarce in the pre-branchial supply to the right kidney. The problem is at least partly overcome by an oxygenated supply to the right kidney from the common aortic trunk and anterior aorta in *Haliotis* whereas there is none to the left (Crofts 1929). This accords well with the view that the left kidney receives oxygenated blood direct from the gill, in the most advantageous position to meet high energy demands for active transport.

Mangum & Polites (1980) have proposed the possibly advantageous operation of a reverse Bohr shift, at pH values below 7.9, which they suggested might prevent excessive depletion of oxygen before the blood reaches the single kidney of *Busycon*. They have overlooked the arterial supply from the posterior aorta normally found in the kidney of monotocardians too, but their argument may still hold and can also be applied to the right kidney of archaeogastropods.

A further advantage is conferred upon the left kidney of an archaeogastropod as a site of active transport in that the urine, as a filtrate of post-branchial blood, will not be laden with nitrogenous excretory products and will have a high pH. In an excretory organ such as the right kidney there is likely to be a high concentration of ammonium ions (discussed below) as demonstrated by Potts (1965) in *Octopus*. This is known to inhibit Na^+-linked transport mechanisms, including the type of glucose resorption demonstrated by Harrison (1962) in *Haliotis*.

While the epithelium of the left kidney is bathed by postbranchial blood on its basal (inner) surface and by a filtrate of the same blood on its luminal surface, that of the right kidney has venous blood on its basal surface and a filtrate of postbranchial blood on its luminal surface, with consequent differences in pH, and solute concentration. This may create conditions in which ammonia diffuses into the lumen of the kidney to be converted into ammonium ions and trapped in the urine. This arrangement, the primitive one in molluscs, favours excretion, but is unsuitable for sodium transport mechanisms and may contribute to the poor regulatory ability of most molluscs compared with gastropods.

Differences in the size of right and left renopericardial canals support the idea that the two kidneys receive and process urine at different rates. Harrison (1962) reported very variable rates of urine production in *Haliotis rufescens*, either one of the two kidneys producing the larger volume at any one time, but without any consistent pattern. This suggests that rates are regulated in the two organs independently according to demand. Since possible neurosecretory granules have been seen in axons of nerves to both kidneys in the three genera studied here, the control mechanism may involve neurosecretion.

Crofts (1929) by contrast, recorded a consistently higher flow of urine from the right kidney

of *H. tuberculata*, despite failing to find a right renopericardial canal and so leaving its source uncertain. A higher rate of flow might be expected in an organ excreting ammonia, though the mantle and gill offer other extensive areas through which it could be lost. On the assumption that these keep ammonia concentrations at acceptable levels, the right kidney, with its large capacity, can store fluid for relatively long periods and act as an accumulation kidney, whereas the left, with its small capacity receives and processes small amounts quickly.

In monotocardians, excretory, regulatory and resorptive functions are all undertaken by the single post-torsional left kidney (figure 32*d*). The dorsal wall of the larger right part of this has the same relationship with the vascular system as the whole right kidney of archaeogastropods, while the nephridial gland has the same relationship as the left, with a direct connection to the auricle. These regions are also the functional counterparts of right and left kidneys respectively. Nevertheless the excretory epithelium over the dorsal wall of the kidney in a monotocardian such as *Littorina* bears unmistakable traces of its origin in a papillary sac (Andrews 1981), though under the influence of its venous blood supply it has assumed again an excretory function lost in the archaeogastropods. Thus, given once more the biochemical environment of the right kidney, it has reacted by acquiring the role of this organ, while the nephridial gland, which retains the same biochemical background as that area in trochids, retains its functions. It would therefore appear that twice during the course of gastropod evolution there has been a change in function of the left kidney in response to biochemical change in its blood supply, and the cells react by doing what this 'allows' them to do.

The single cell type of the archaeogastropod papillary sac is therefore replaced over the dorsal wall of the monotocardian kidney by two types, one being the ciliated resorptive type characteristic of a papillary sac which persists virtually unchanged, the second and by far the more abundant, being derived from the former but specialized for nitrogenous excretion (Andrews 1981).

The second type, in accordance with Dollo's Law, is a monotocardian innovation not a reversion to the primitive condition and is the homologue of papillary sac epithelium, not the excretory epithelium of the right kidney of archaeogastropods. The reported differences between excretory cells of archaeogastropods and monotocardians (Andrews 1981) are therefore only to be expected. This interpretation also offers an explanation for the variability of excretory cells in different groups of monotocardians, since they probably arose independently along several different evolutionary lines.

The origin of the nephridial gland itself remains obscure. Its absence in pleurotomariaceans is believed to be primitive but its occurrence in unrelated archaeogastropods from very different habitats is difficult to understand. It is placed in the most favourable situation in the system for active transport, where postbranchial blood appears continuously to move over the epithelium basally, in concert with the heart-beat. Its existence may, therefore have contributed to colonization of the littoral zone by some trochids confronted with the attendant problem of regulating the ionic composition of the blood. The appearance of a nephridial gland in such a distantly related and archaic type as *Neomphalus* is less easy to understand. However, this animal too, lives in a difficult environment, close to hot sulphur springs at great depths (Fretter *et al.* 1981) where some regulatory capacity may be essential in maintaining the correct composition of body fluids. There are clearly similarities between the thickened part of the left kidney in *Patella* and the nephridial gland of other prosobranchs, in that there are features associated with active transport, but the differences are sufficiently great to suggest that the

specializations have arisen after the Patellacea diverged from the rest of the gastropod stock. Again it may be correlated with an intertidal mode of life in these limpets.

In all these instances it is the same region of the kidney, dictated by its relationship to other structures which has specialized and to this extent the 'nephridial gland' appears to be homologous in the three groups, but it seems more likely that it has been a separate innovation in each. The monotocardian nephridial gland, however, does appear to be the homologue of its trochid counterpart.

The peculiarities of both kidneys of the Patellacea strengthen the argument of Salvini-Plawen (1980) that patellaceans are set apart from other archaeogastropods and must have separated from the rest of the gastropod stock at a very early evolutionary stage. The kidneys of the Fissurellacea, by contrast, are fundamentally similar at the ultrastructural level to those of the pleurotomariacean–trochacean line suggesting that they have diverged more recently.

The physiological implications of the near loss of the left kidney in fissurellaceans are not difficult to reconcile with their mainly sublittoral habit and their limited tolerance of emersion or lowered salinity (Fretter & Graham 1976). This is not so for the Patellacea, however, with their ability to tolerate the vagaries of an intertidal habitat and salinities as low as 25% in natural conditions. While the ultrastructure of the left kidney suggests a resorptive capacity, its relatively small size casts doubt on its ability to have sole responsibility for solute regulation. The large capacity of the right kidney may provide a reservoir of fluid and it too may have a role in resorption. It may be significant, however, that two sections of the very long intestine, designated A and B by Graham (1932) show ultrastructural features associated with transporting epithelia (M. Bush, personal communication). The possibility therefore exists that the intestine compensates for the reduction of the left kidney by supplementing its role in regulation of body fluids.

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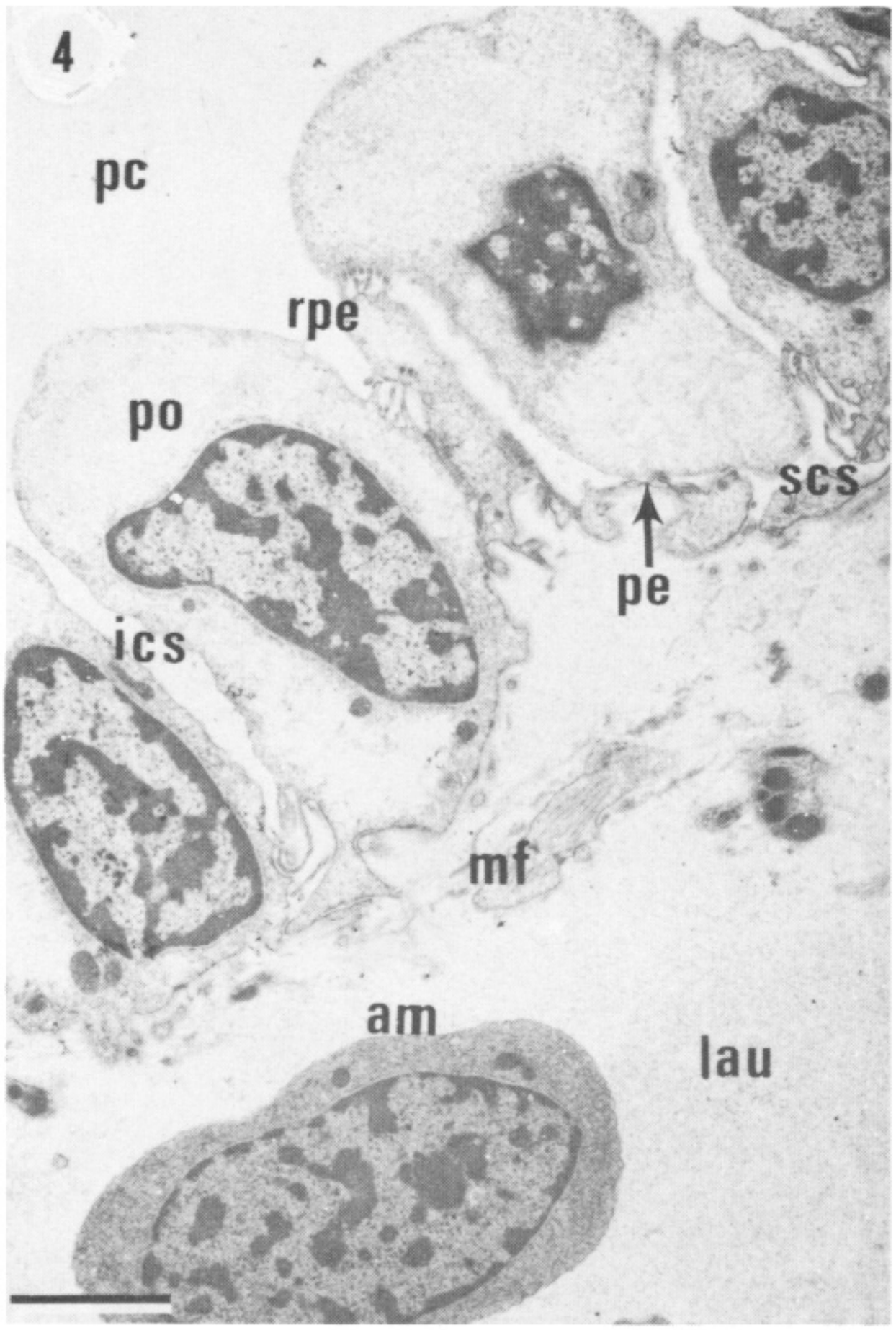
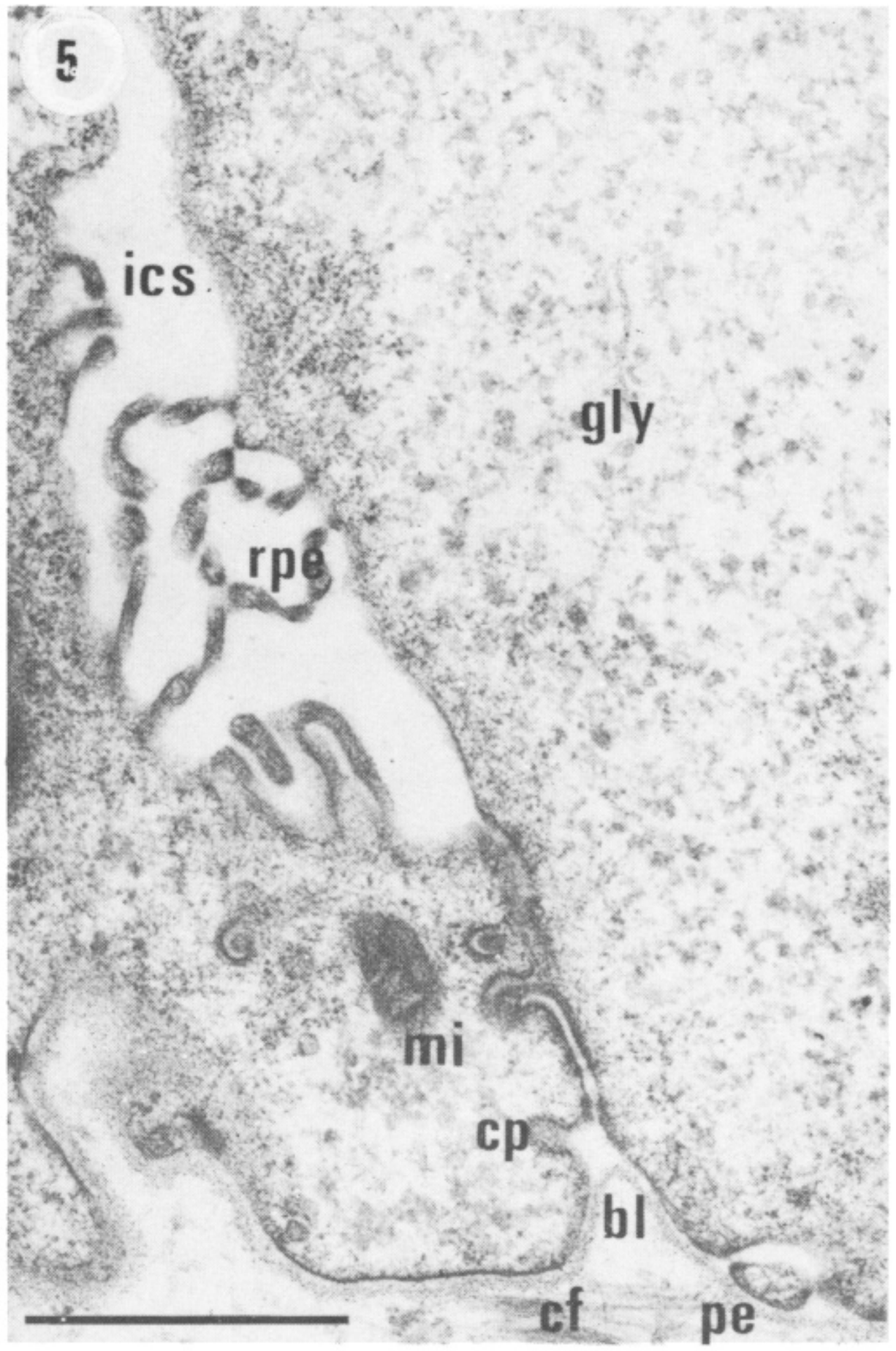
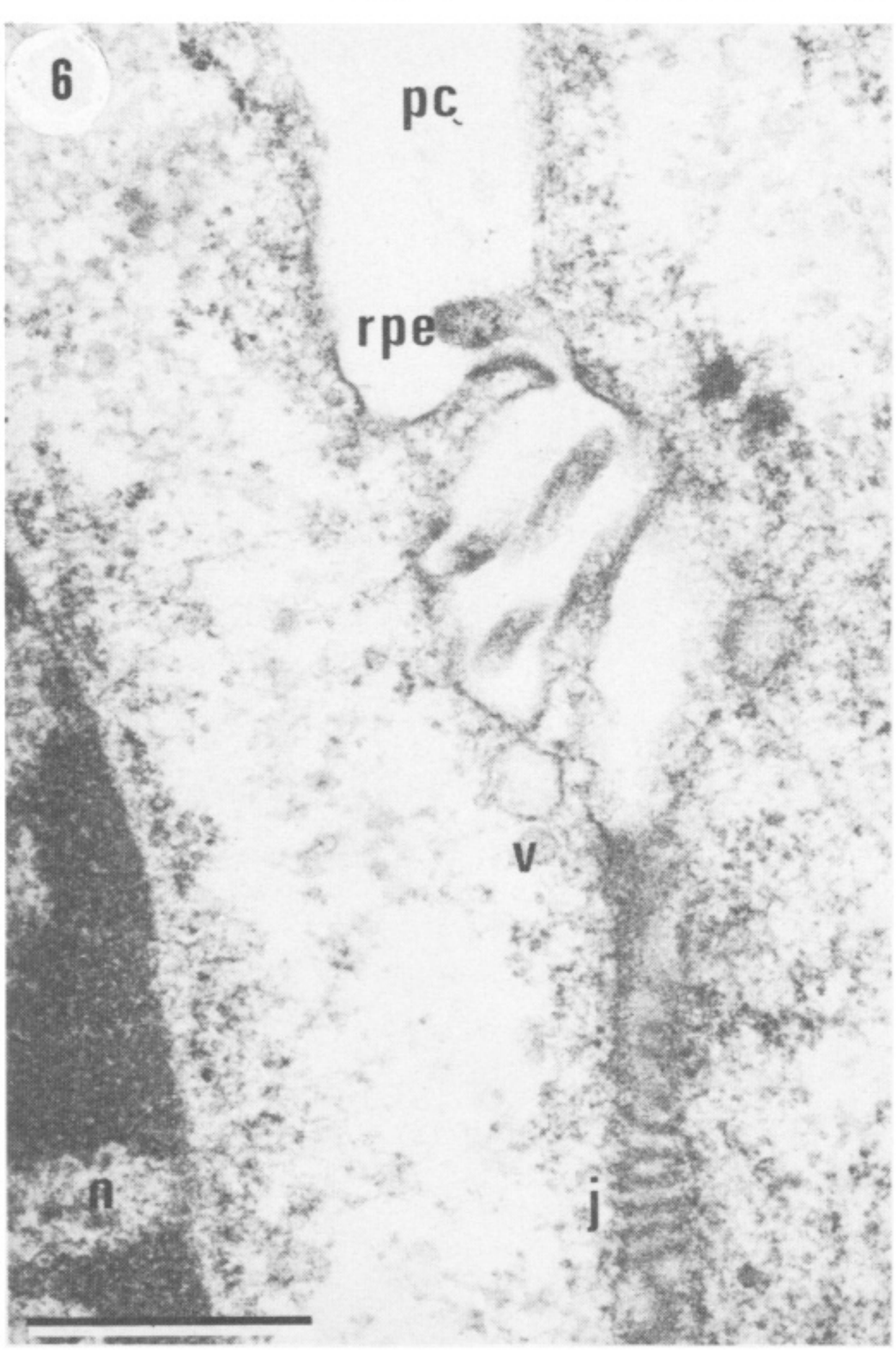
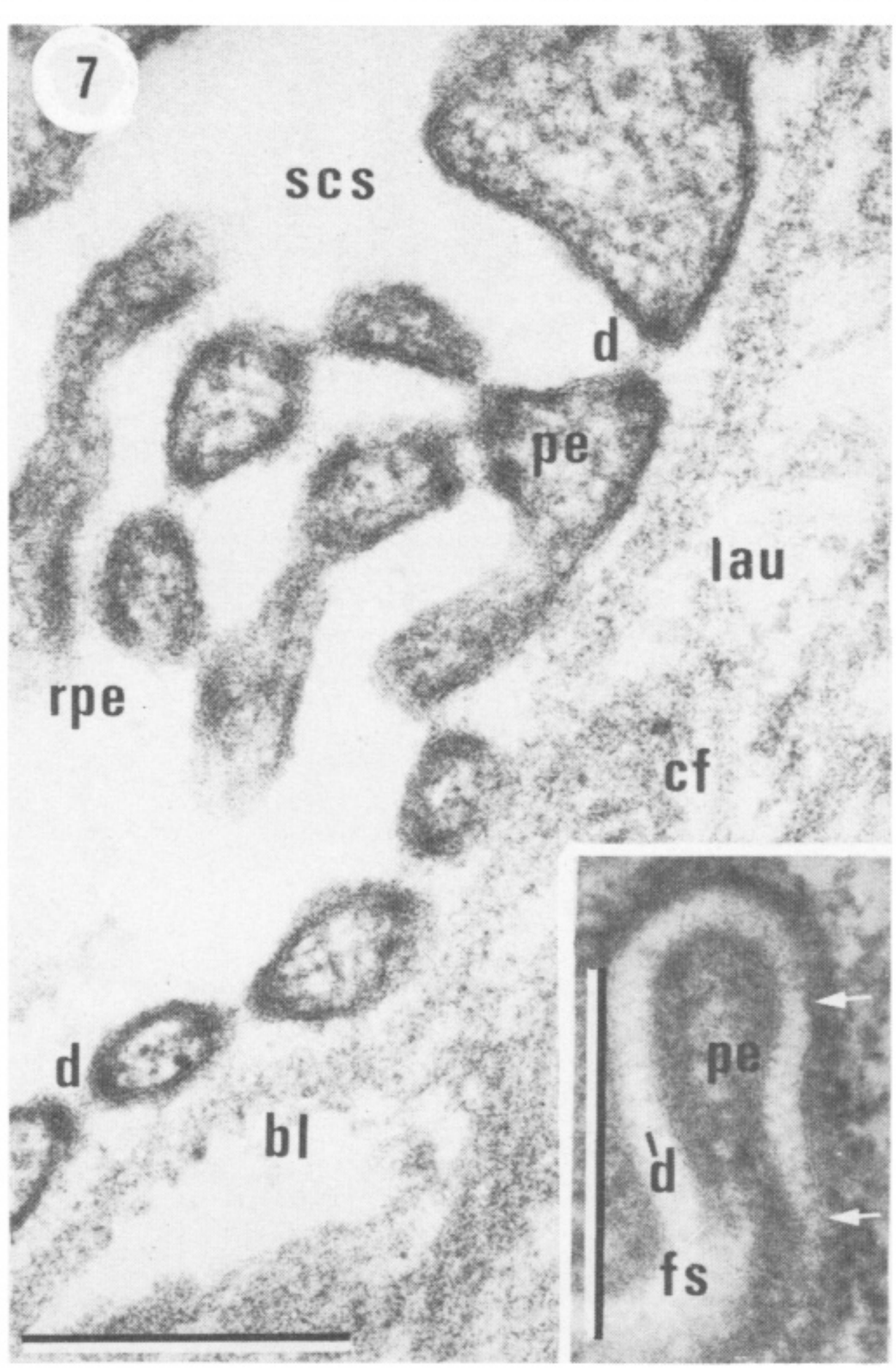
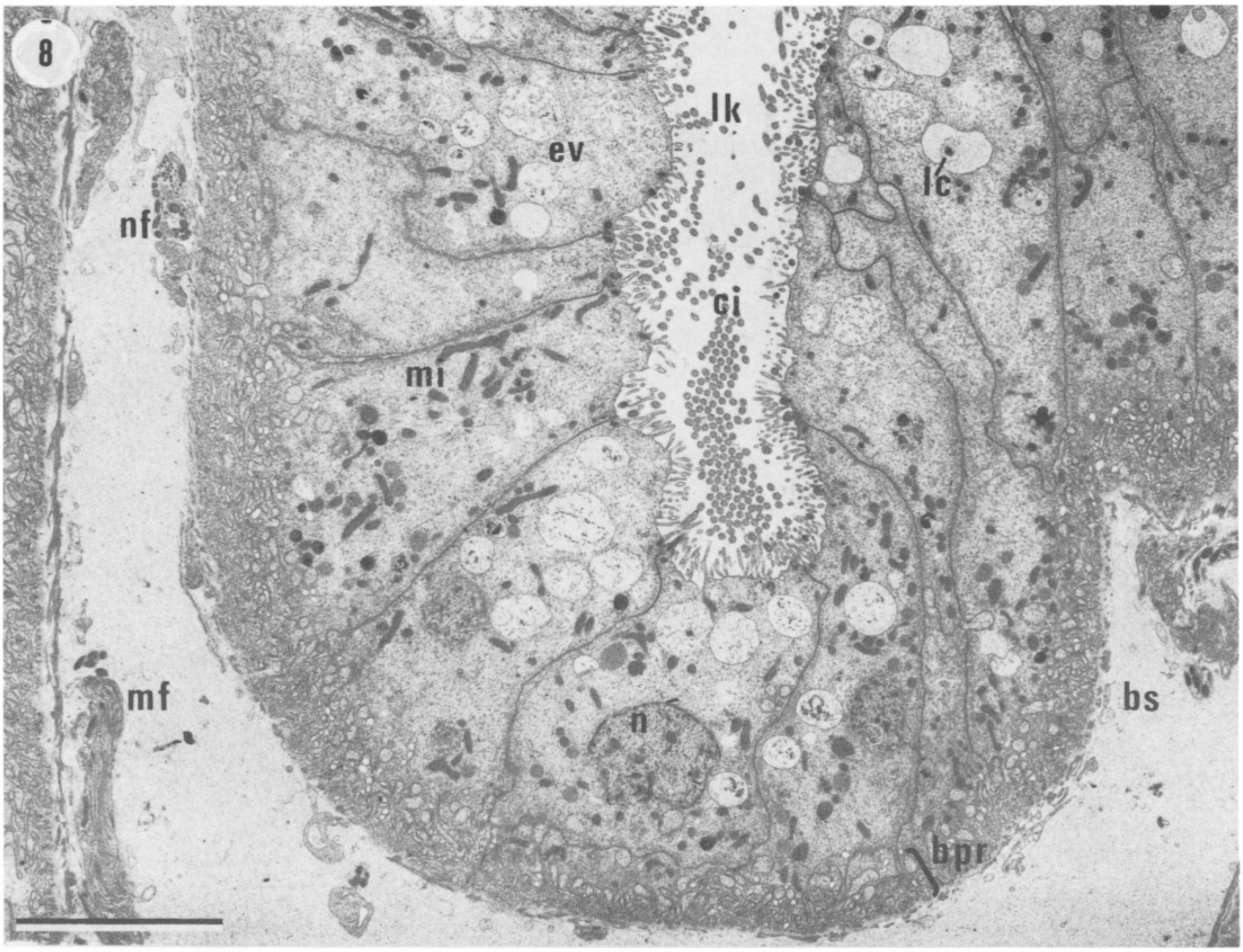


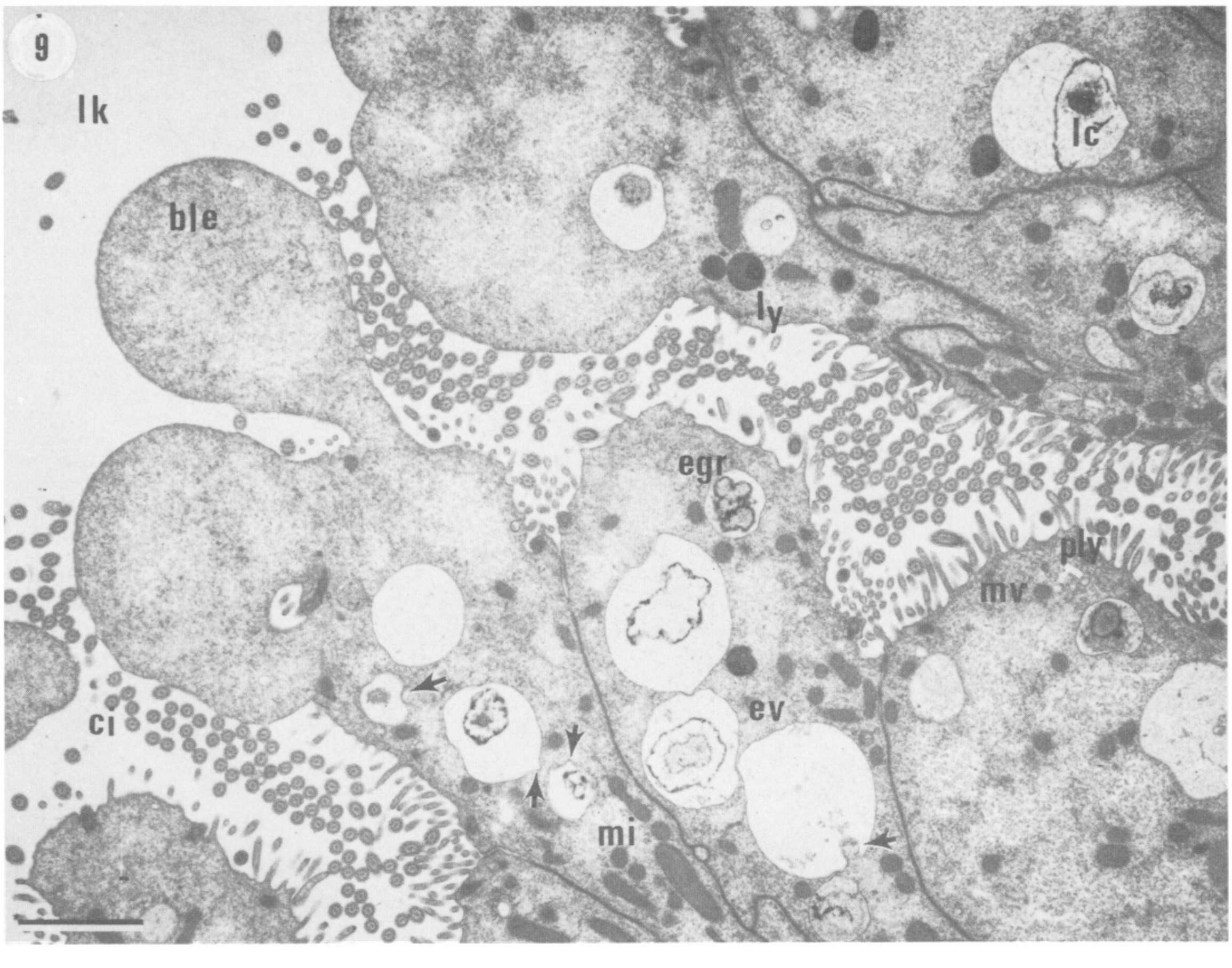






FIGURES 4–7. For description see opposite.





FIGURES 8 AND 9. The right kidney of *Monodonta*, proximal region.

FIGURE 8. Part of the cuboidal epithelium. Scale bar, 10 μm.

FIGURE 9. The apical regions of excretory cells during a phase when blebs of cytoplasm are being nipped off. Scale bar, 2 μm. ble, Blebs of cytoplasm; bpr, basal processes; bs, blood space; ci, cilia; egr, excretory granule; ev, excretory vacuole; lc, lipofuscin core; lk, lumen of kidney; ly, lysosome; mf, muscle fibre; mi, mitochondrion; mv, microvilli; n, nucleus; nf, nerve fibre; ply, primary lysosome.

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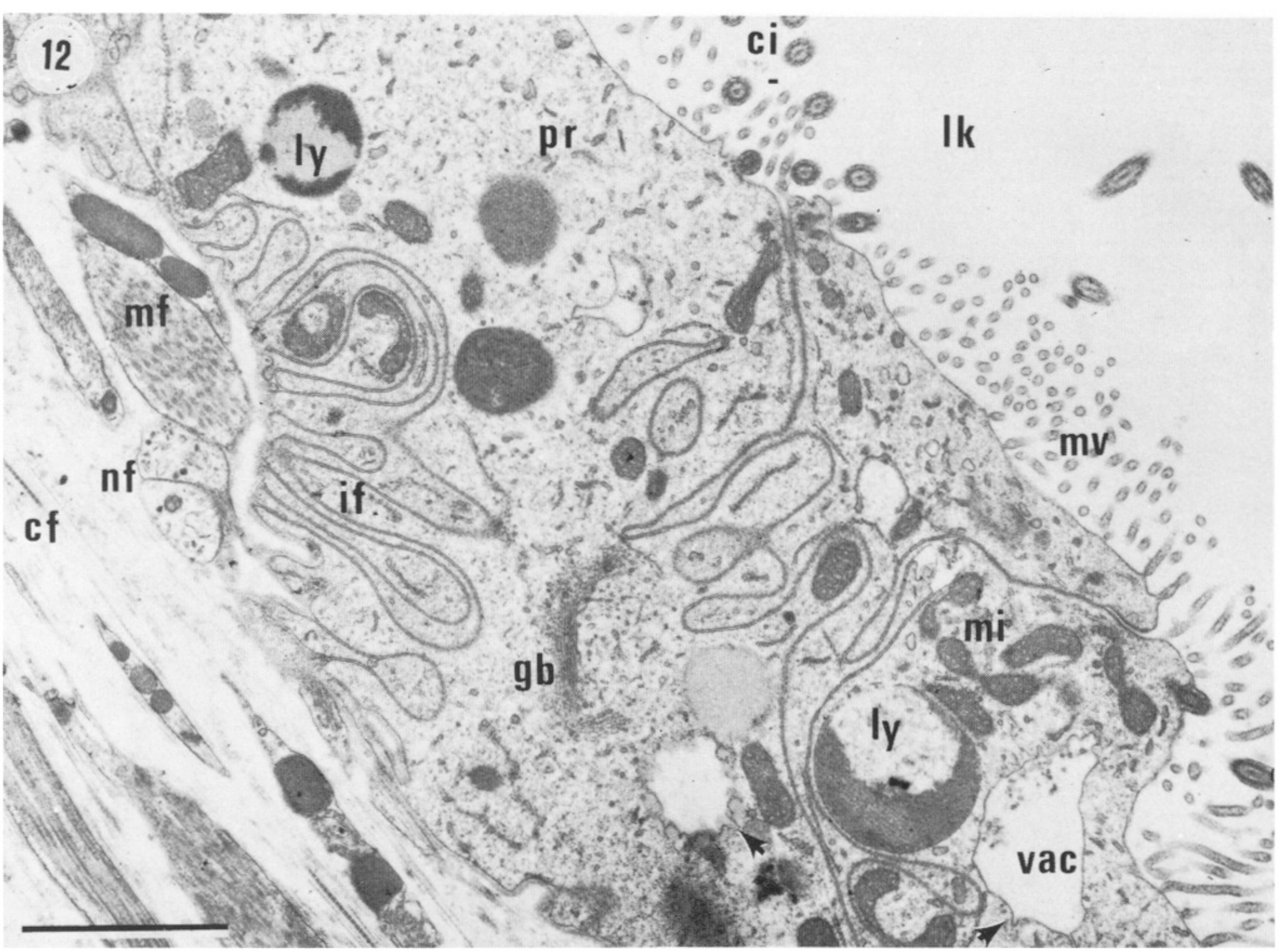
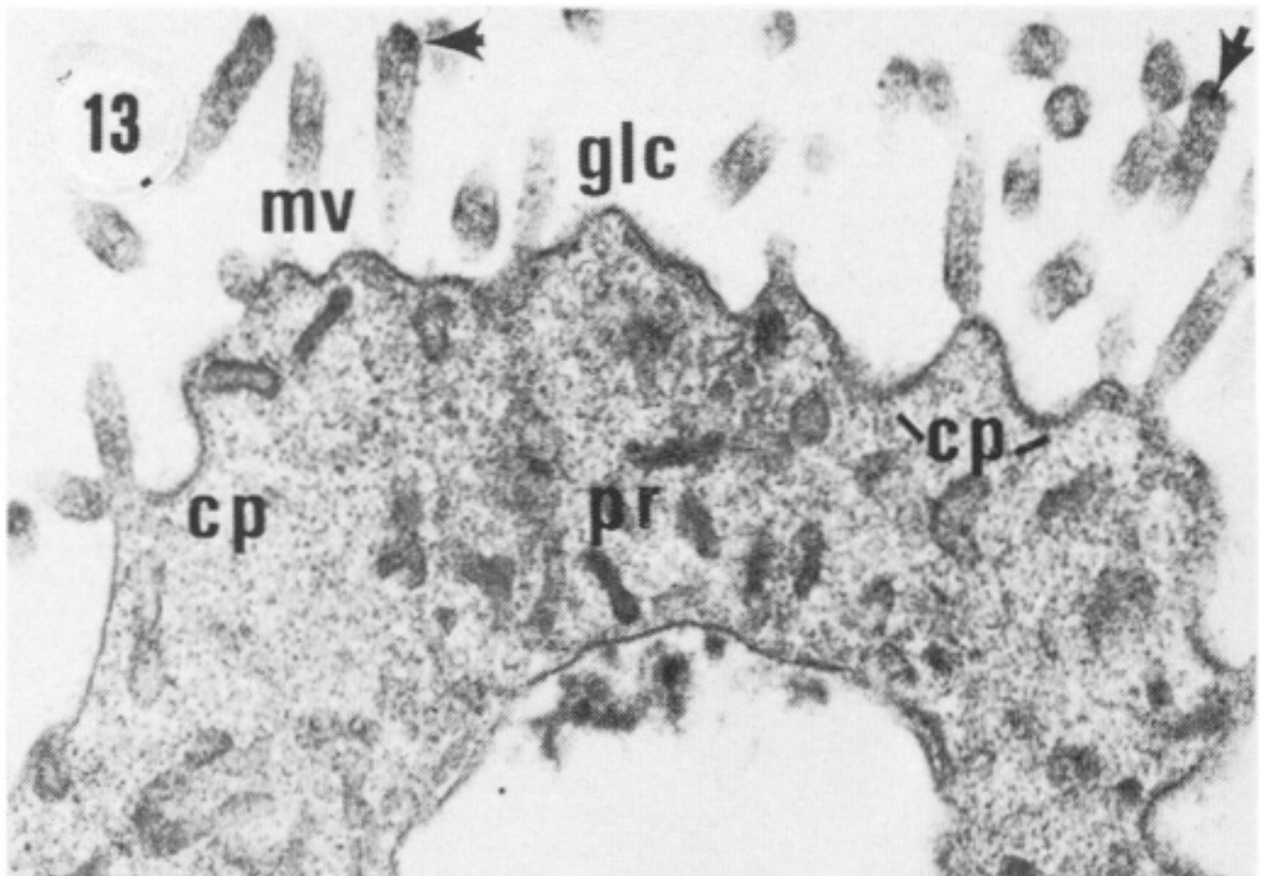
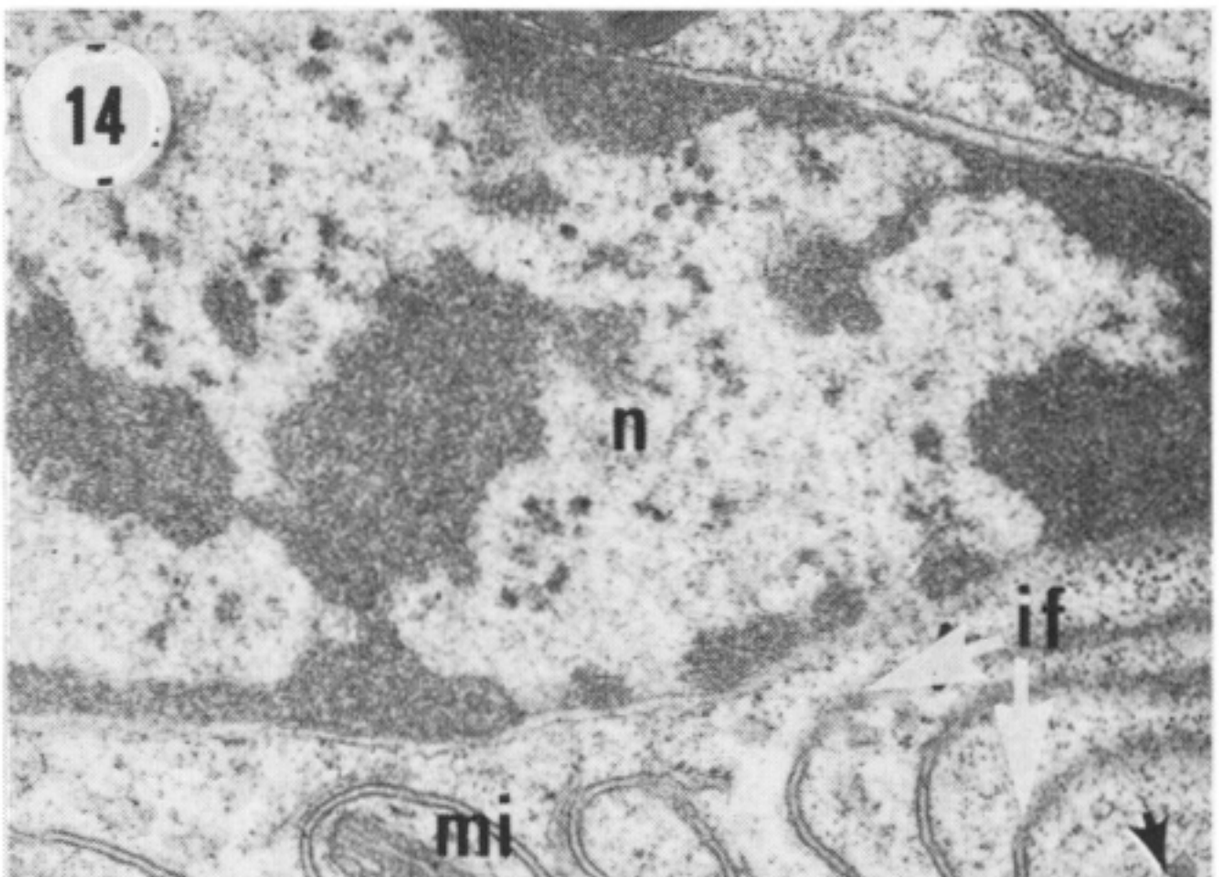
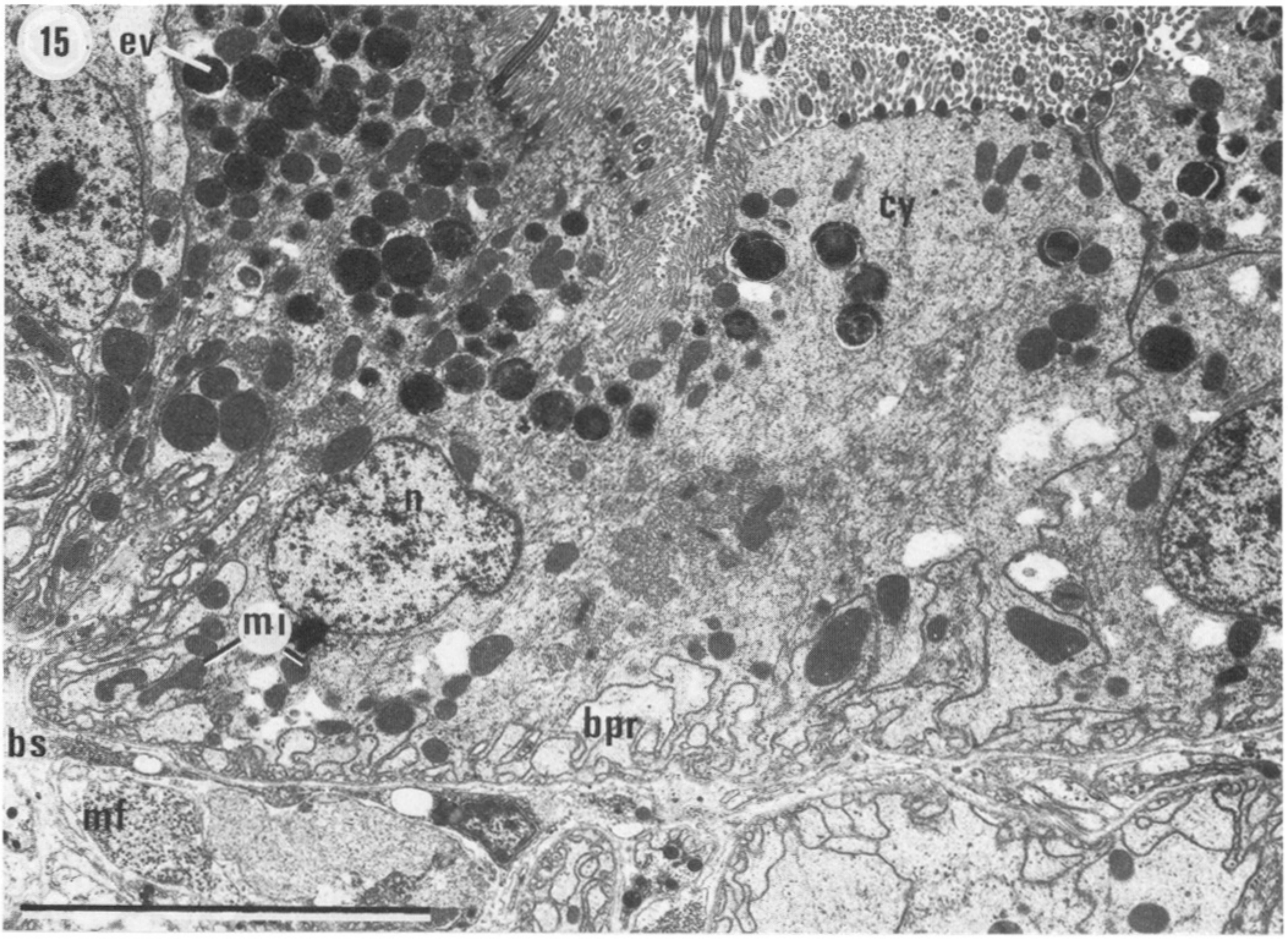
FIGURES 10 AND 11. For description see p. 394.

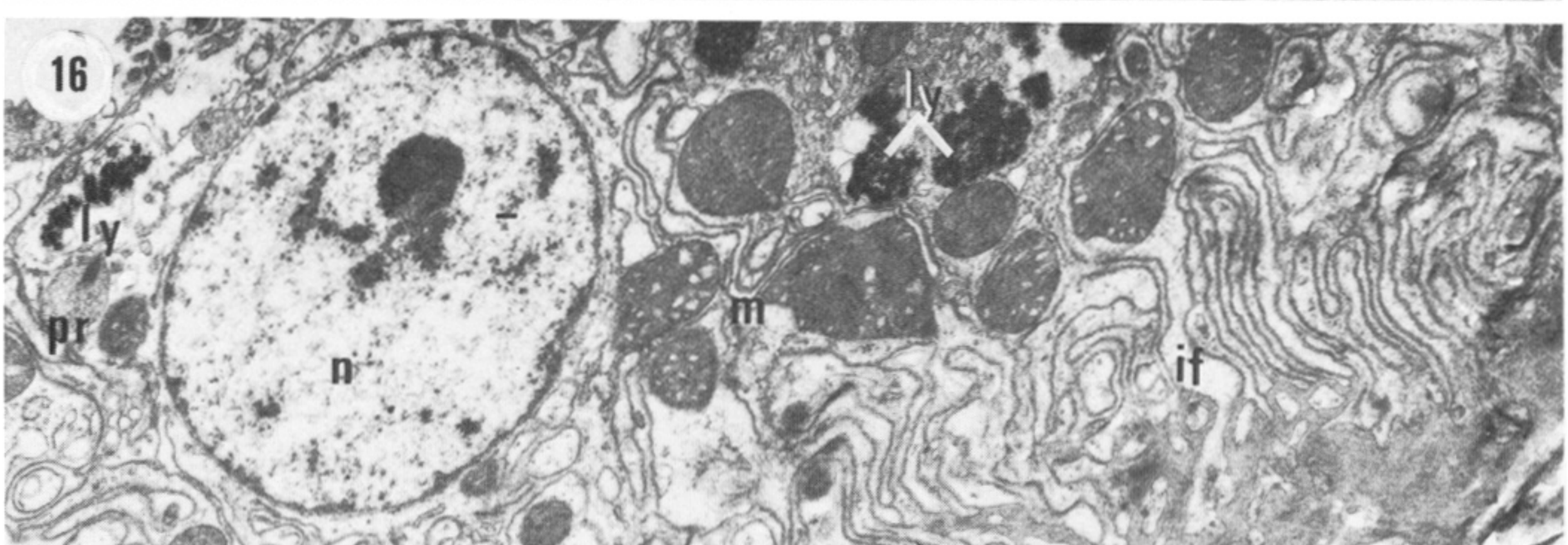




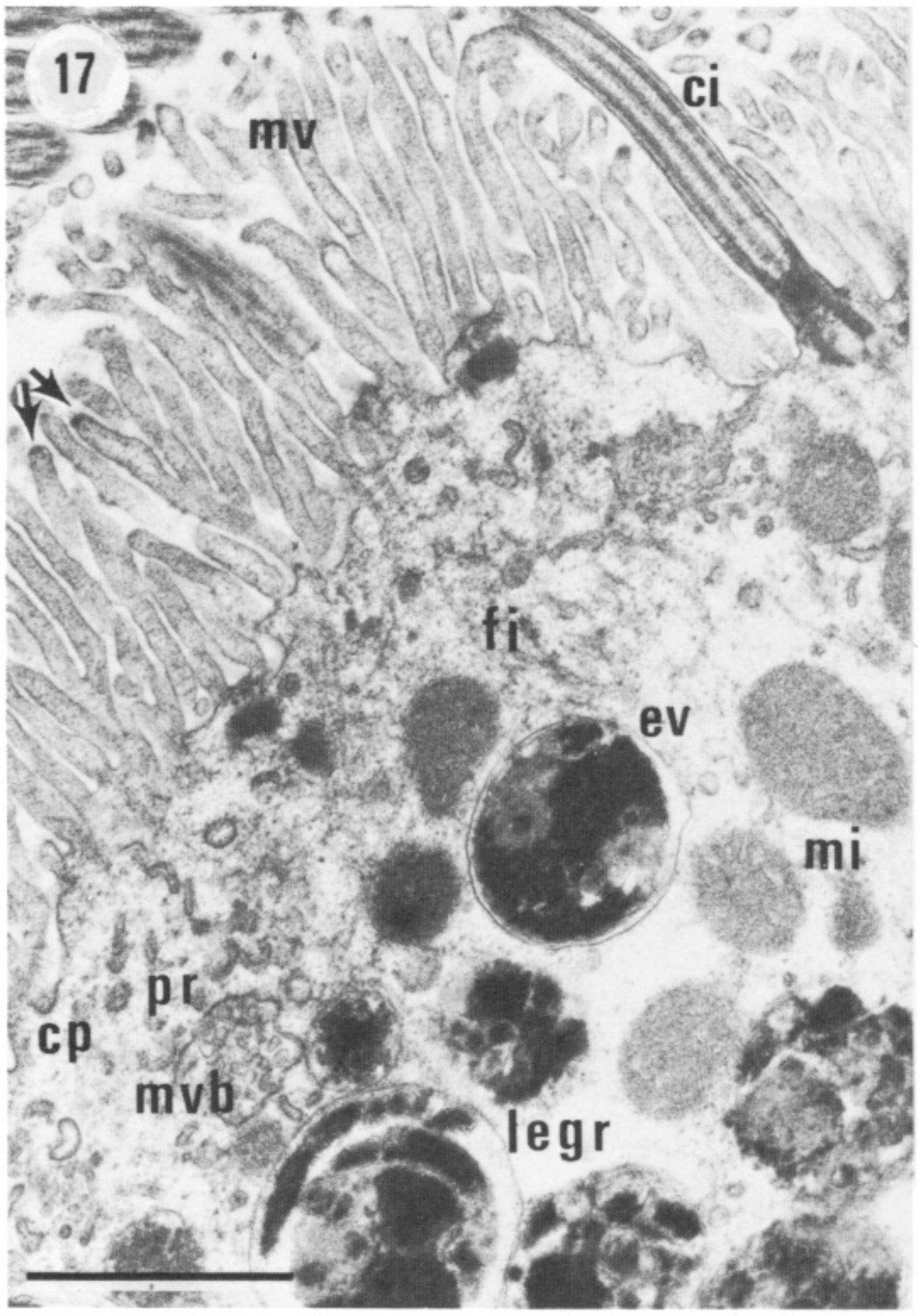


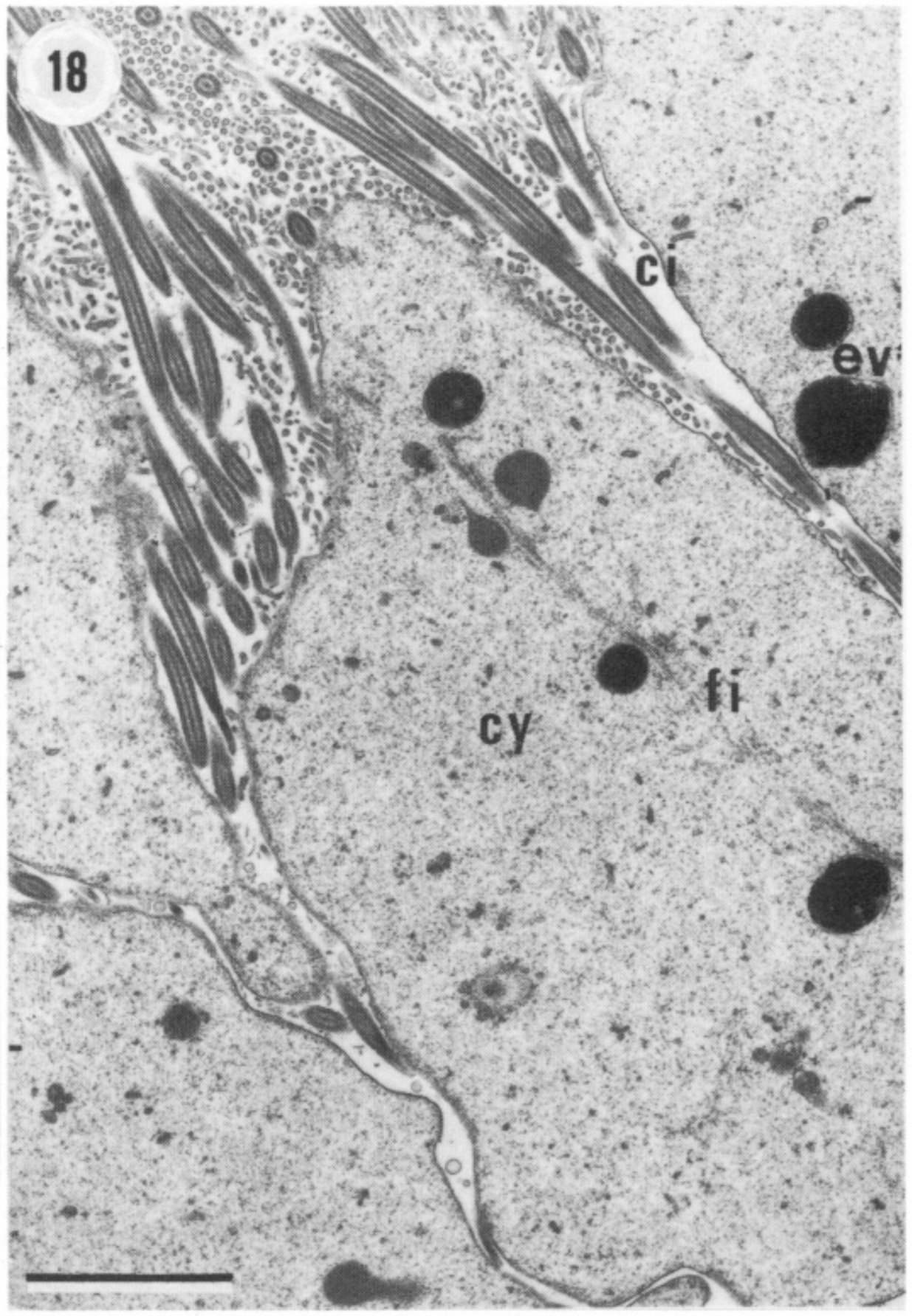
FIGURES 12–14. For description see opposite.

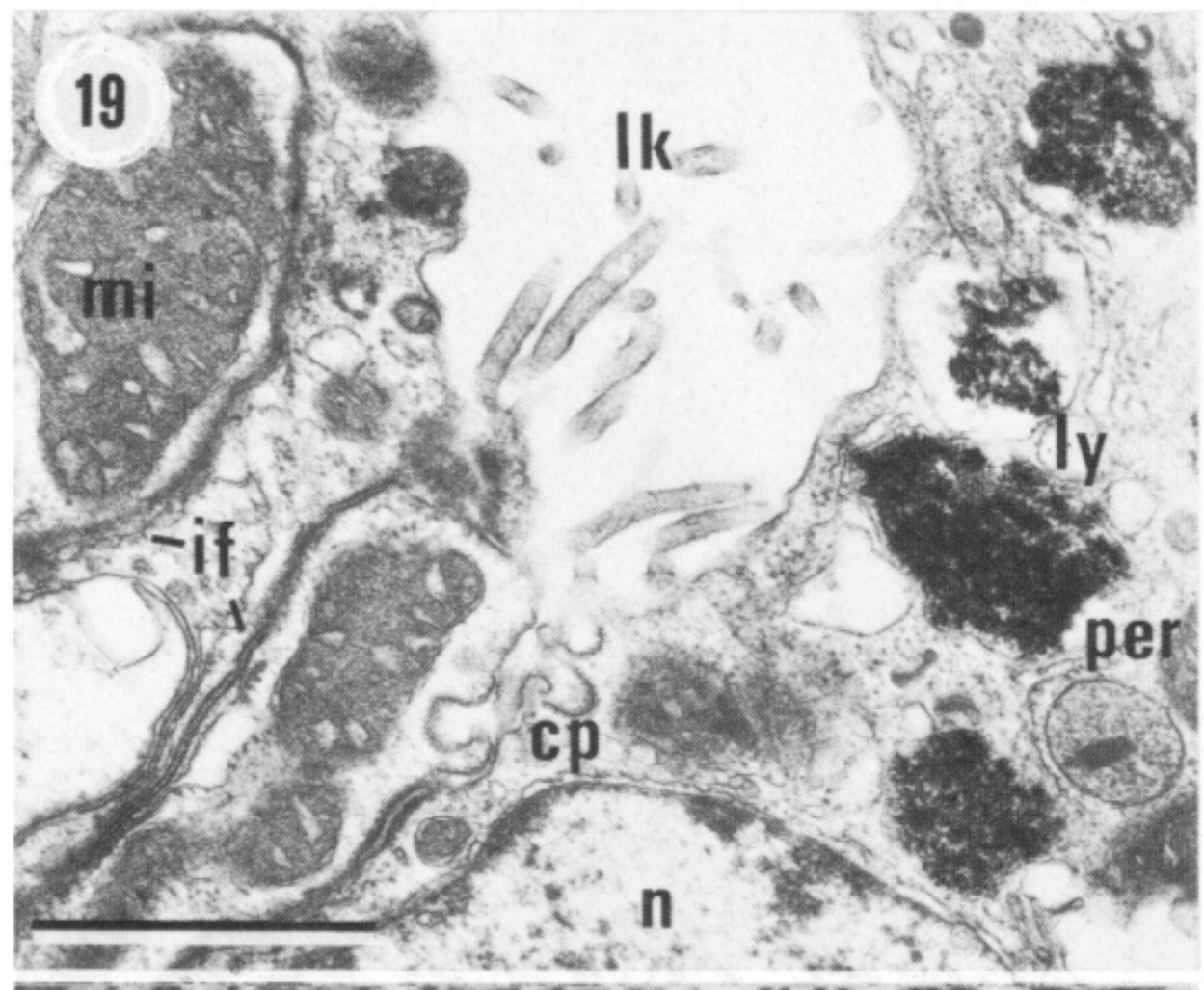


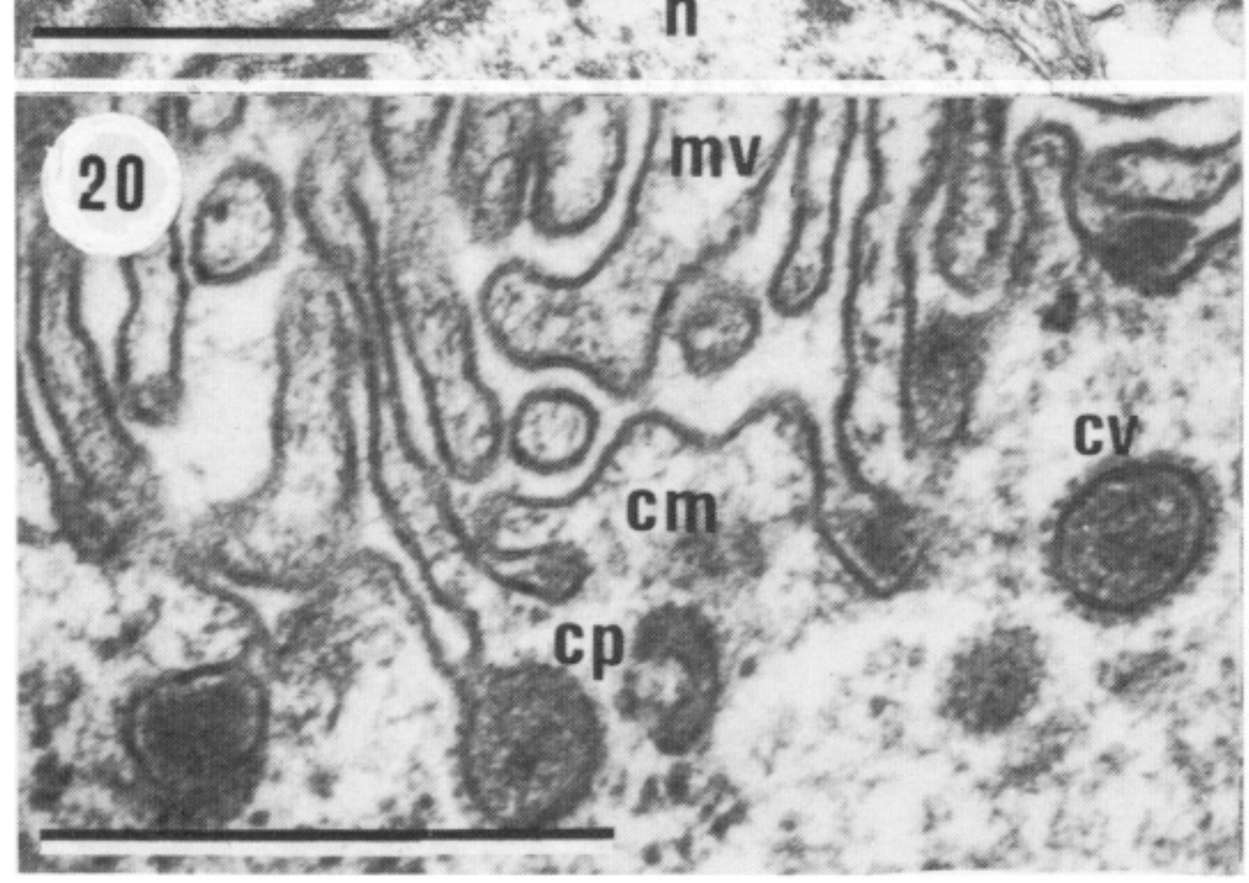


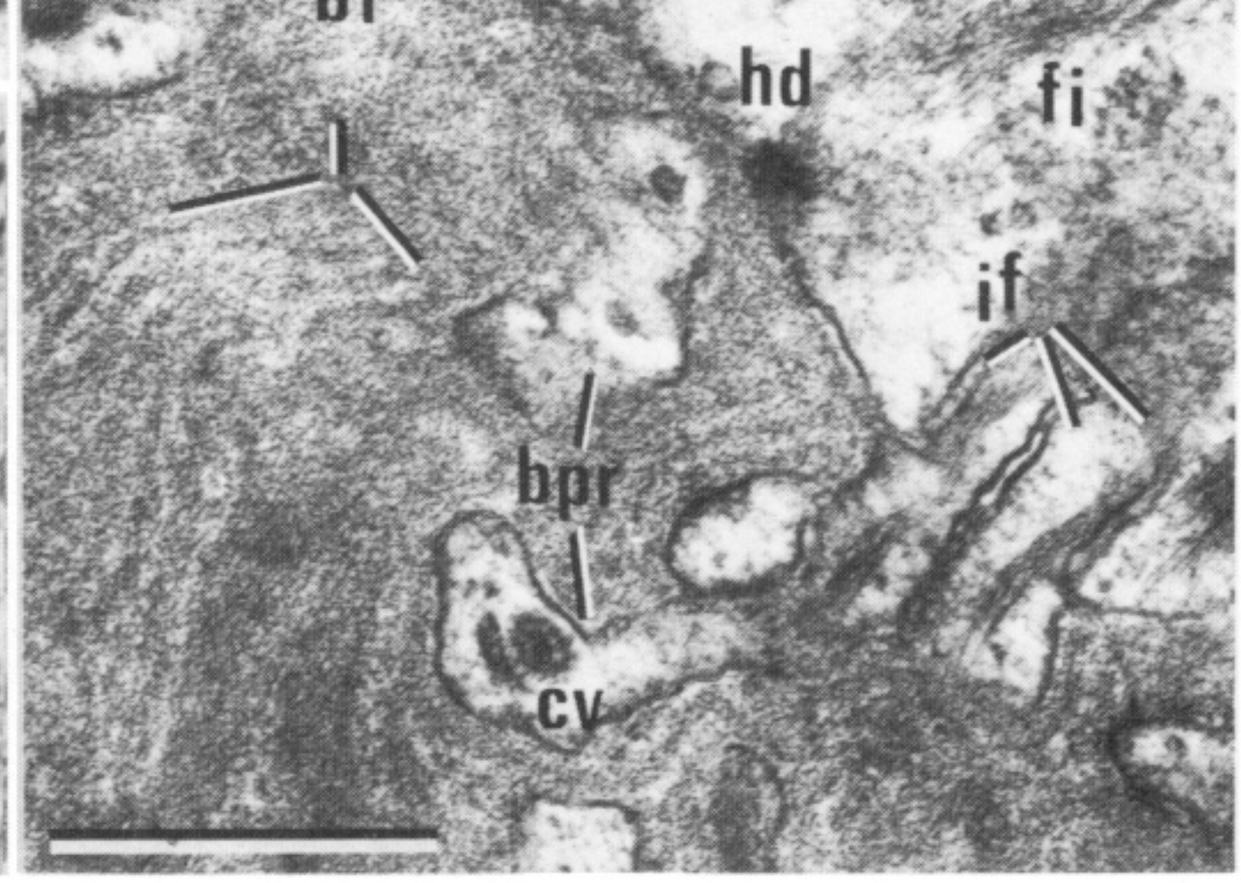
FIGURES 15 AND 16. For description see opposite plate 4.



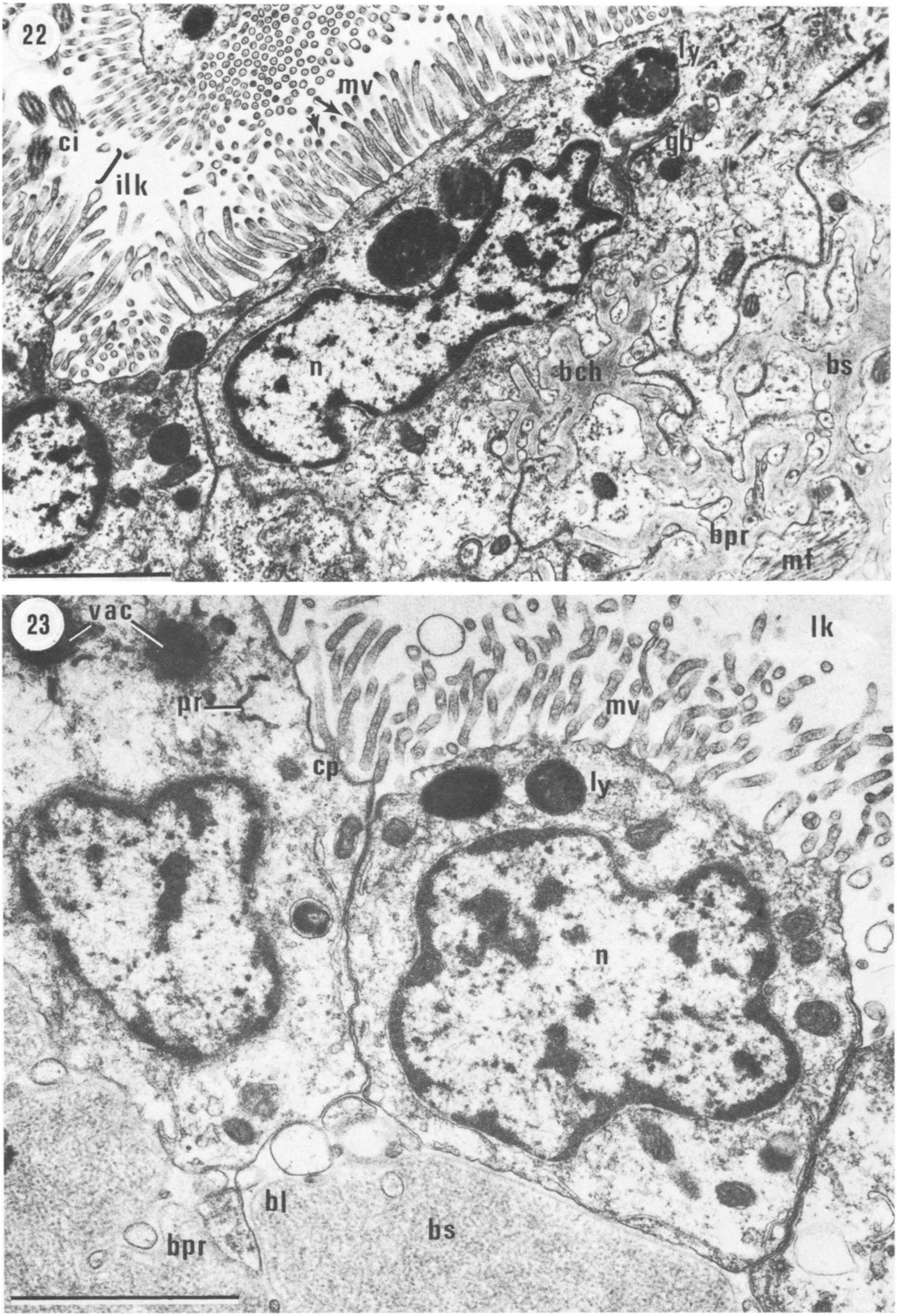




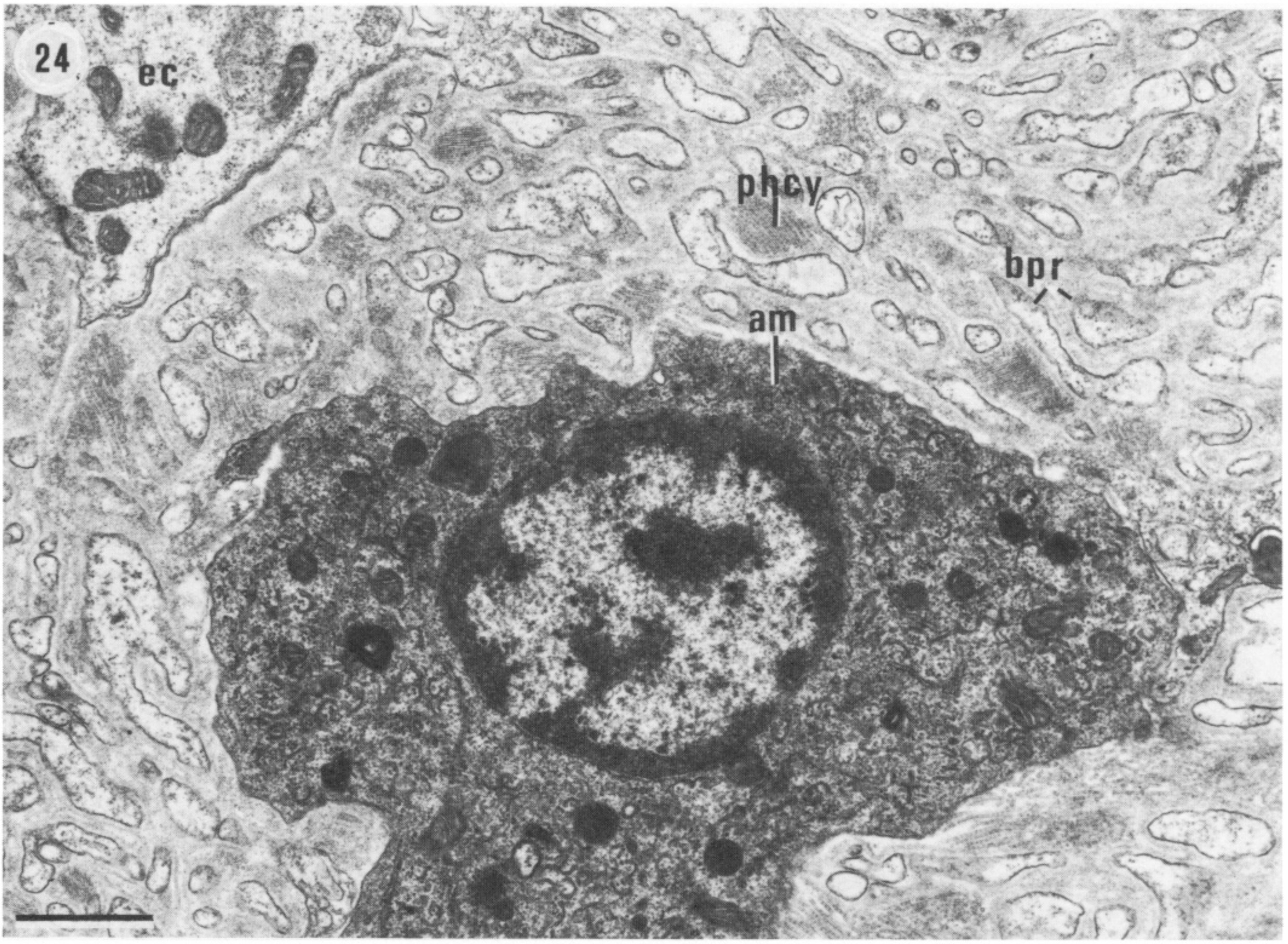


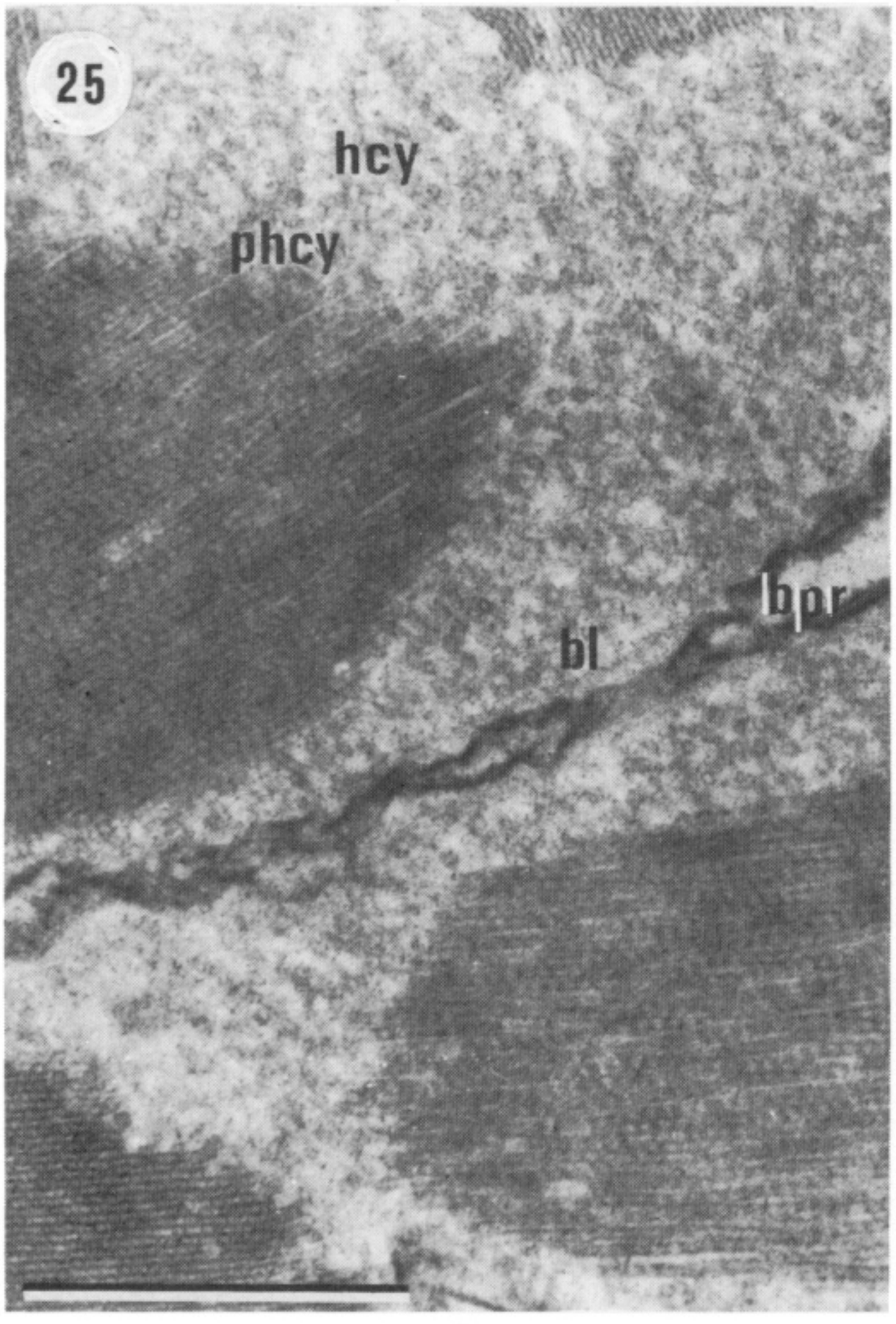


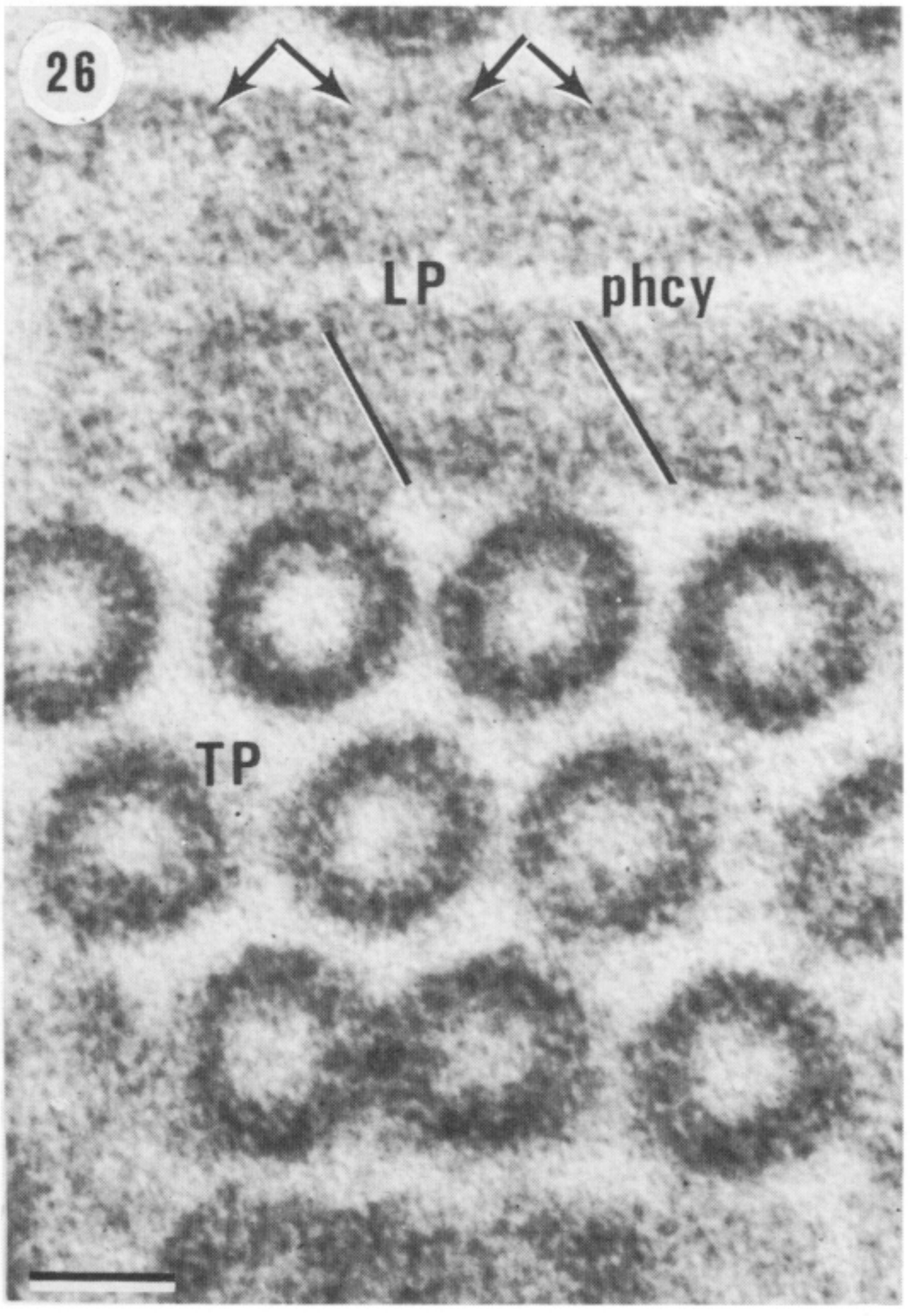
FIGURES 17–21. For description see opposite plate 7.



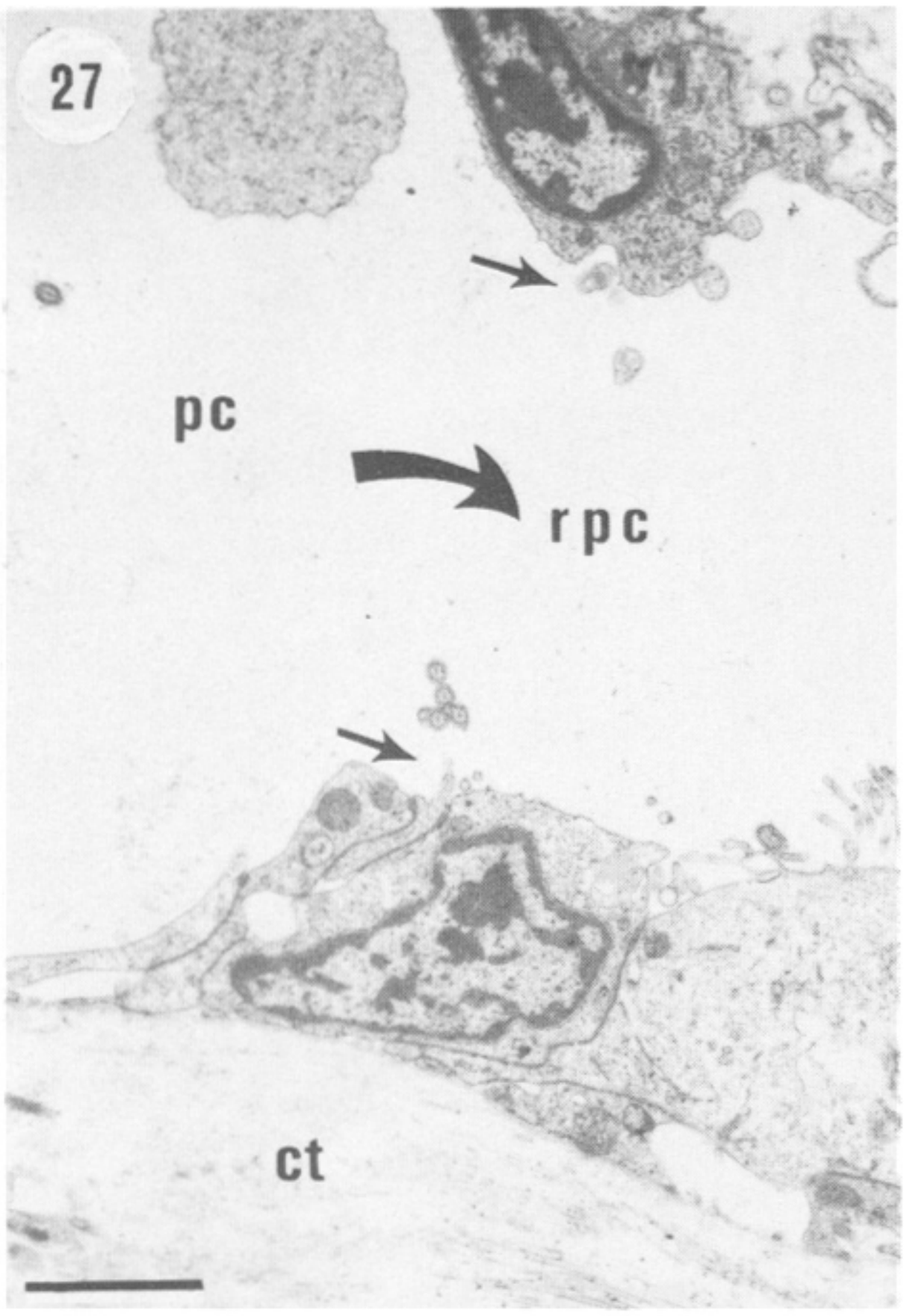
FIGURES 22 AND 23. For description see opposite.

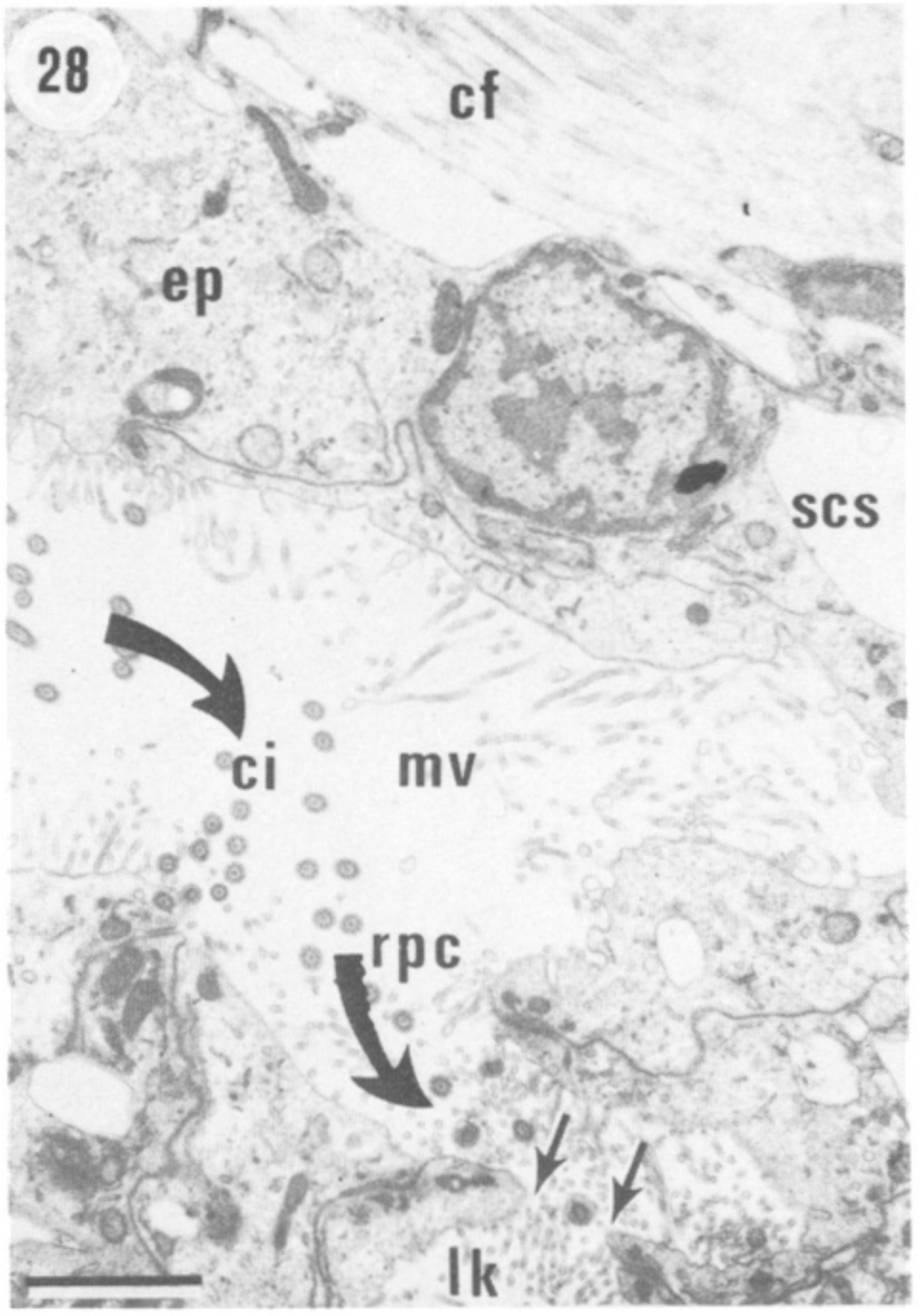


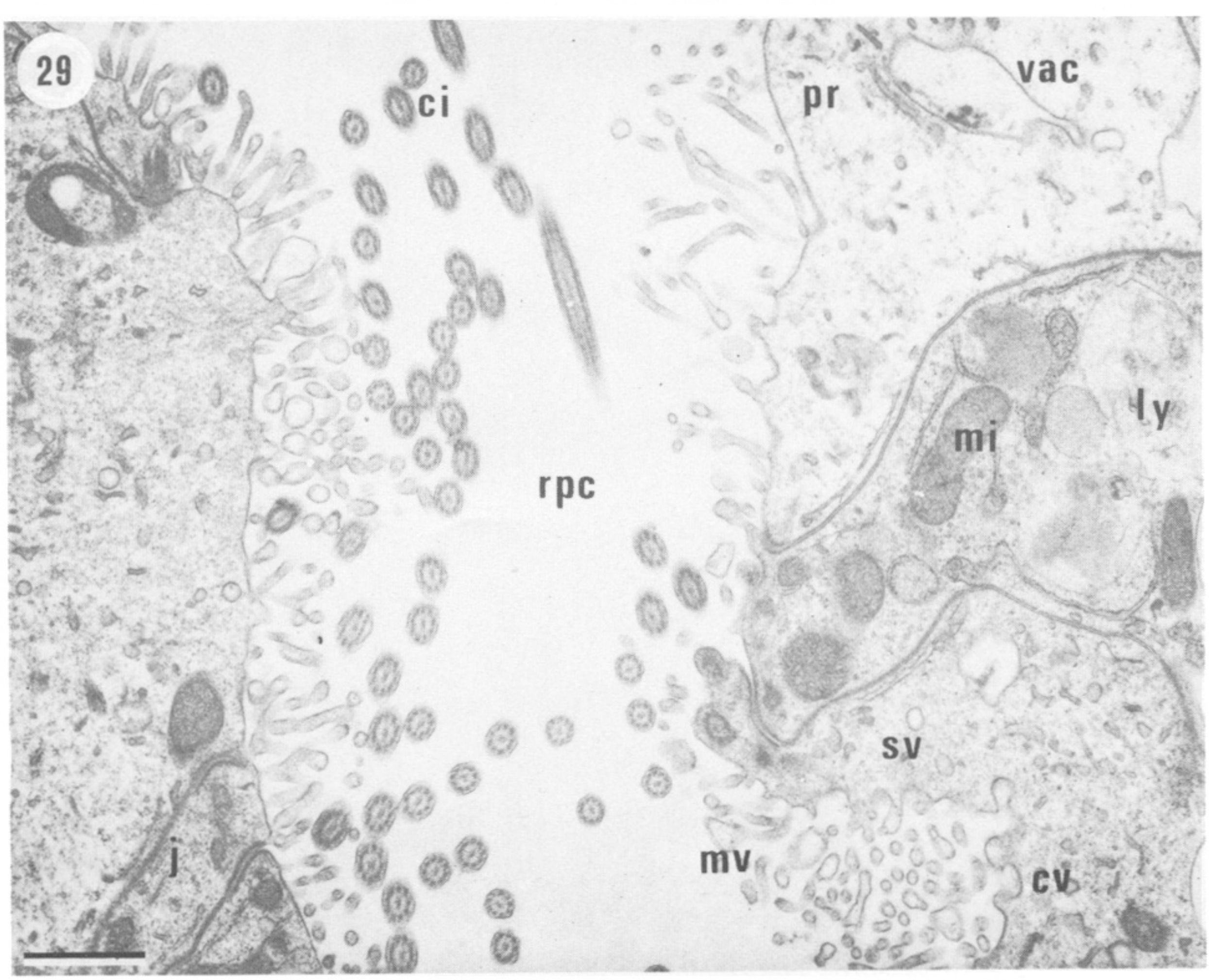




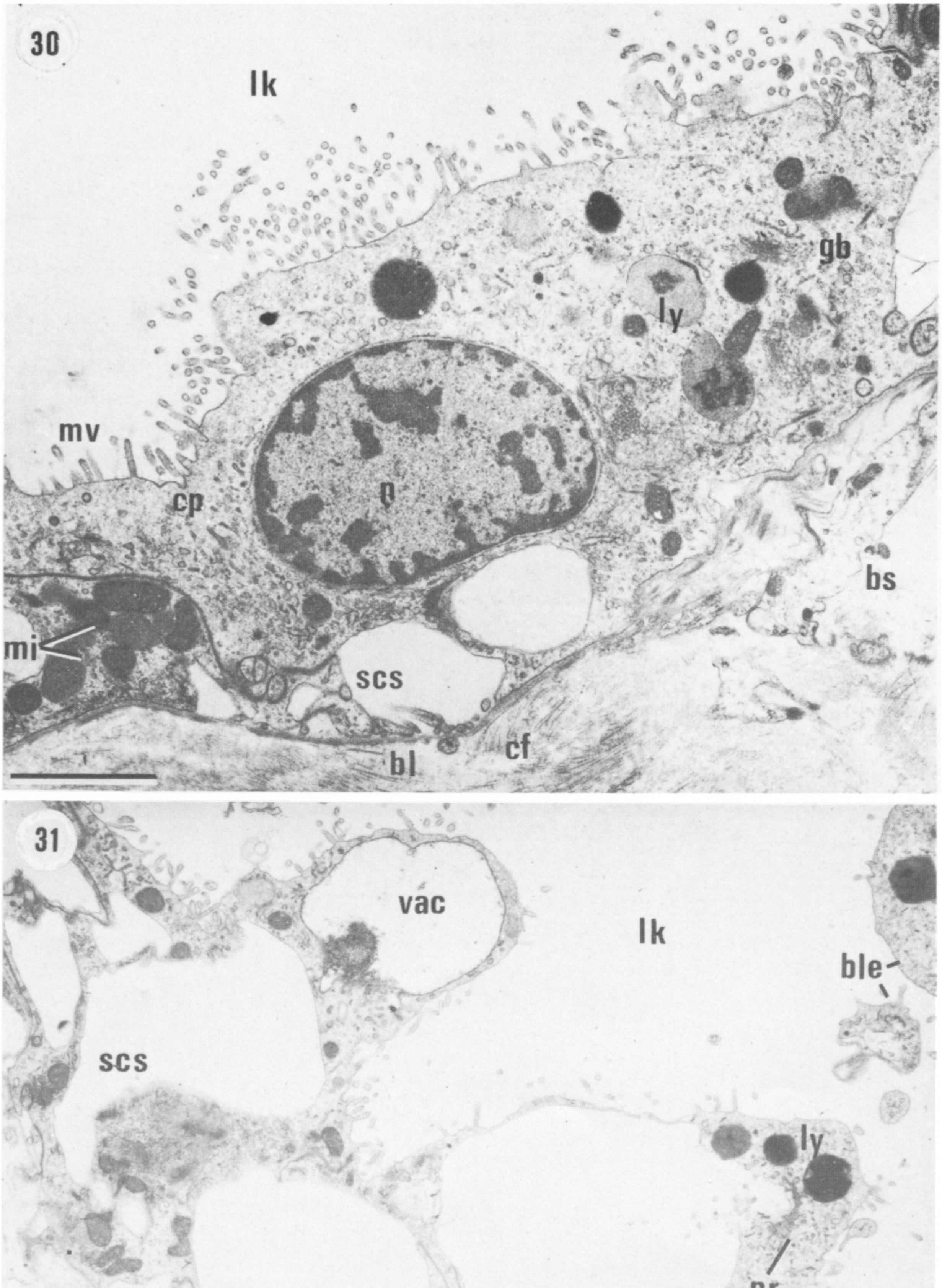
FIGURES 24–26. For description see p. 395.







FIGURES 27–29. For description see p. 395.



FIGURES 30 AND 31. For description see opposite.