ORGANIZATION OF THE OUTER SYNAPTIC LAYER IN THE RETINA OF THE LARVAL TIGER SALAMANDER

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CONTENTS

The organization of the outer synaptic layer in the salamander retina was studied electronmicroscopically in serial sections of tissue prepared by conventional techniques or stained by the method of Golgi. Rod cell pedicles make ribbon junctions on cone cell processes, and rod cell processes invaginate cone pedicles without otherwise making any specialized contact with them. Horizontal cells make ribbon and distal junctions with the photoreceptor cell pedicles; a single horizontal cell may contact both rods and cones. Bipolar cells were observed to make either ribbon or basal junctions with the photoreceptor cell pedicles; in addition, certain processes believed to belong to bipolar cells make both ribbon and basal junctions with the same or different pedicles. A single bipolar cell may make contact with both rods and cones. Horizontal cells synapse on bipolar cell dendrites and on certain unidentified processes which in turn are also presynaptic to bipolar cells. Ascending branches of these processes invaginate deeply the rod and cone pedicles without otherwise engaging them in any junction. Horizontal cell processes are linked by two kinds of junctions: close membrane appositions, and contacts analogous to the distal junctions between horizontal cells and rod pedicles.

Introduction

Recent observations in the turtle retina revealed an unexpected variety of specialized contacts at cone pedicles (Lasansky 1971). Horizontal cells make proximal and distal junctions with the pedicles; as implied by the terms, these junctions are found at different locations, and their structure also differs significantly. Processes believed to belong to certain bipolar cells are flanked by horizontal cell endings at assemblies known as 'triads', and engage also in two kinds of junctions: apical and distal. In addition, the cone pedicles make basal junctions with bipolar cells, symmetrical junctions with adjacent pedicles, and unidentified junctions at distant pedicles.

Available studies on other retinas do not make reference to such a complex organization at rod or cone endings. It seemed of interest, therefore, to test the general validity of the findings on turtle cones by extending the observations to other vertebrate photoreceptors. The retina of the tiger salamander was chosen for this purpose because its neurons are relatively large (diameter of the somata 12 to 16 μm), an important feature should electrophysiological studies with microelectrodes become desirable.

As shown by the following account, rod and cone pedicles in the retina of the salamander display a variety of specialized contacts matching that found in turtle cones. This study includes also observations on contacts between second-order neurons, and some basic patterns of neuronal organization at the outer synaptic layer are described.

Methods

All the observations were performed on larvae of the tiger salamander, *Ambystoma tigrinum tigrinum*. Larvae, rather than adult animals, were used because of their availability throughout most of the year. Specimens 10–14 cm long were obtained from a commercial supplier (Mogul-Ed, Oshkosh, Wisconsin, U.S.A.), and usually sacrificed a day after arrival in the laboratory.

The eyes were excised under ordinary illumination and sectioned at the equator; the posterior hemisphere was fixed for 2 to 3 h in an ice-cold mixture of 2.5 g/100 ml glutaraldehyde and 1 g/100 ml OsO_4 (Simionescu, Simionescu & Palade 1972) in 0.1 M phosphate buffer (pH 7.4). The glutaraldehyde–OsO_4 mixture was adopted after repeated failures to obtain adequately fixed retinas with the same fixatives used either alone or consecutively. Glutaraldehyde (with or without formaldehyde) followed by OsO_4 – a procedure that gave good fixation when used for the turtle retina (Lasansky 1971) – resulted in an unacceptable proportion of fragmented membranes, vacuolized cytoplasms, and myelin figures. On the other hand, OsO_4 alone –used by other authors (Dowling 1968; Dowling & Werblin 1969) for studies on amphibian retinas– although giving a generally good preservation of membranous structures, caused excessive extraction of cytoplasmic materials, thus resulting in the loss of identifying cellular features such as the different density of cone and rod cytoplasms (see below). The glutaraldehyde–OsO_4 mixture showed the virtues, but none of the drawbacks, of both fixatives, without introducing any noticeable structural changes, except with regard to the shape of the rod and cone synaptic vesicles (see below). All the cell junctions described in the following show the same structural detail irrespective of whether glutaraldehyde and OsO_4 are used simultaneously or consecutively.

After the glutaraldehyde–OsO_4 fixation, the retinas were cut into small pieces, and postfixed for 2 h in 2 g uranyl acetate per 100 ml maleate buffer (Karnovsky 1967). Following dehydration in graded ethanol, the tissue was embedded in Epon (Luft 1961).

When serial sections were used, they were mounted on 1 mm × 2 mm slot grids coated by carbon and formvar films. Ten series of 70 to 146 sections were examined and photographed at a magnification of × 5400. To follow cell processes in consecutive serial sections, they were identified on the basis of their intrinsic appearance and relation to neighbouring elements, the latter determined visually or by means of measurements. Segments of some of the series will be shown in the following when a particular point can be illustrated with a reasonably small number of micrographs. Isolated sections mounted on conventional grids were also studied and photographed at magnifications of × 2600 to × 42000 in a Siemens Elmiskop 1A. All the sections were stained with lead citrate (Reynolds 1963).

Golgi-stained retinas were prepared in a similar manner to that described earlier (Lasansky 1971). The tissue was fixed as indicated above, and then soaked for 2 to 3 days in a solution of 0.3 g/100 ml OsO_4 in 2.7 g/100 ml potassium dichromate, and for 1 to 2 days in 0.8 g/100 ml silver nitrate. The last two steps were repeated once or twice. This procedure gave the highest yield of stained cells among several other variants, but the results were still unsatisfactory, the main problem being the tendency of the cells to stain in compact clusters.

Because of the frequent contamination by other stained elements, only a few of the Golgi-stained cells were judged suitable for electron microscopic observation. The procedure followed is the one introduced by Stell (1965), and applied before to the turtle retina (Lasansky 1971). Only cells stained on a clean background were selected for this purpose, but as an additional precaution, serial sections were obtained and mounted on slot grids, as recommended by Kolb (1970). In this way, the stained processes could be traced to thicker and recognizable portions of the chosen cells, so that their origin could be ascertained beyond doubt.

Observations

Morphology of rod and cone endings

The photoreceptor cells of the salamander are single and double cones, red rods, and green rods (Walls 1963). In ordinary histological preparations, cone cell nuclei are found to be adjacent to the synaptic region, while rod cell nuclei are more externally placed. As a consequence, cone cell perikarya are continuous with the pedicles, while rod cell perikarya are linked to the pedicles by means of a fibre (figure 1, plate 47) (Ramón y Cajal 1933). Some rod cells exhibit two or three pedicles connected to the perikaryon by separate fibres (figure 5, plate 47), or two pedicles in series linked by a tangential process (figure 4).

Cone and rod pedicles give origin to basal processes (figures 1 to 3, plate 47). Some of them, usually one to three per pedicle, extend horizontally for 15 to 20 μm, have a beaded profile, and appear to end within the outer plexiform layer (figure 2). Others are directed vitread and seem to end at the outer margin of the inner nuclear layer (figure 3).

The cone pedicles can be easily identified at the electron microscope level because of the proximity of the cell nucleus (figure 22, plate 50; figure 23, plate 51.) Once this initial identification has been made, they can usually be distinguished from rod pedicles because the latter have a more opaque cytoplasmic matrix and larger synaptic vesicles (figure 12, plate 49; figures 22, 23). Measurements performed in cells that had round vesicles (see below) indicated a diameter of 35 to 45 nm for cone vesicles and 45 to 50 nm for rod vesicles. Similar differences between cone and rod vesicles have been already reported in the frog and chick retinas (Evans 1966).

Under the present conditions of fixation, the synaptic vesicles at rod pedicles, and less frequently at cone pedicles, showed flattening to a degree that varied among different pedicles and from one block of tissue to another. No attempt was made to investigate the conditions that result in more consistent and pronounced flattening, except to note that it did not occur when glutaraldehyde preceded OsO_4 fixation. Since this is the fixation procedure used by other workers in studies on flat synaptic vesicles (Gray 1969), the finding of flat rod and cone vesicles is not readily comparable to analogous observations at synaptic endings elsewhere in the nervous system.

Some of the rod pedicles are connected to a vertical fibre (figure 23, plate 51), while others are accessory pedicles connected to the main ones by a tangential process, as mentioned above (figure 4, plate 47). Serial sections showed that each cone pedicle is surrounded by 4 to 5 rod pedicles, and this group is separated from similar groups by thick Müller cell processes. Cone pedicles are less frequently found to be adjacent to one another, and in such instances it is likely that the pedicles belong to the two elements of double cones.

Types of specialized contacts at rod and cone pedicles

Ribbon junctions

Ribbon junctions† are defined by the presence in the adjacent photoreceptor cell cytoplasm of an opaque organelle known as the synaptic lamella or ribbon (Ladman 1958). In all instances previously reported, the ribbons were found bisecting wedge-shaped projections of the pedicles, the synaptic ridges. This is also the prevalent arrangement, but not the only one (see below) in salamander visual cells. The synaptic ridges may be seen in any given plane of section to

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Description of plate 47

Figure 1. Rod (RC) and cone (CC) cells stained by the method of Golgi. A fibre (f) links the rod pedicle to the perikaryon, while the cone pedicle and perikaryon form a single body. Basal processes (bp) originate from the pedicles. The outer portions of both cells are masked by silver deposits because the photoreceptor cell layer was directly exposed to the staining solutions. Well-impregnated photoreceptor cells were observed only under such conditions, that is, when detached retinas were processed. (Magn. × 800.)

Figure 2. Golgi-stained cone cell (CC) in the salamander retina. The pedicle gives origin to a basal process (bp) which extends horizontally and ends with a small swelling (Magn. × 800.)

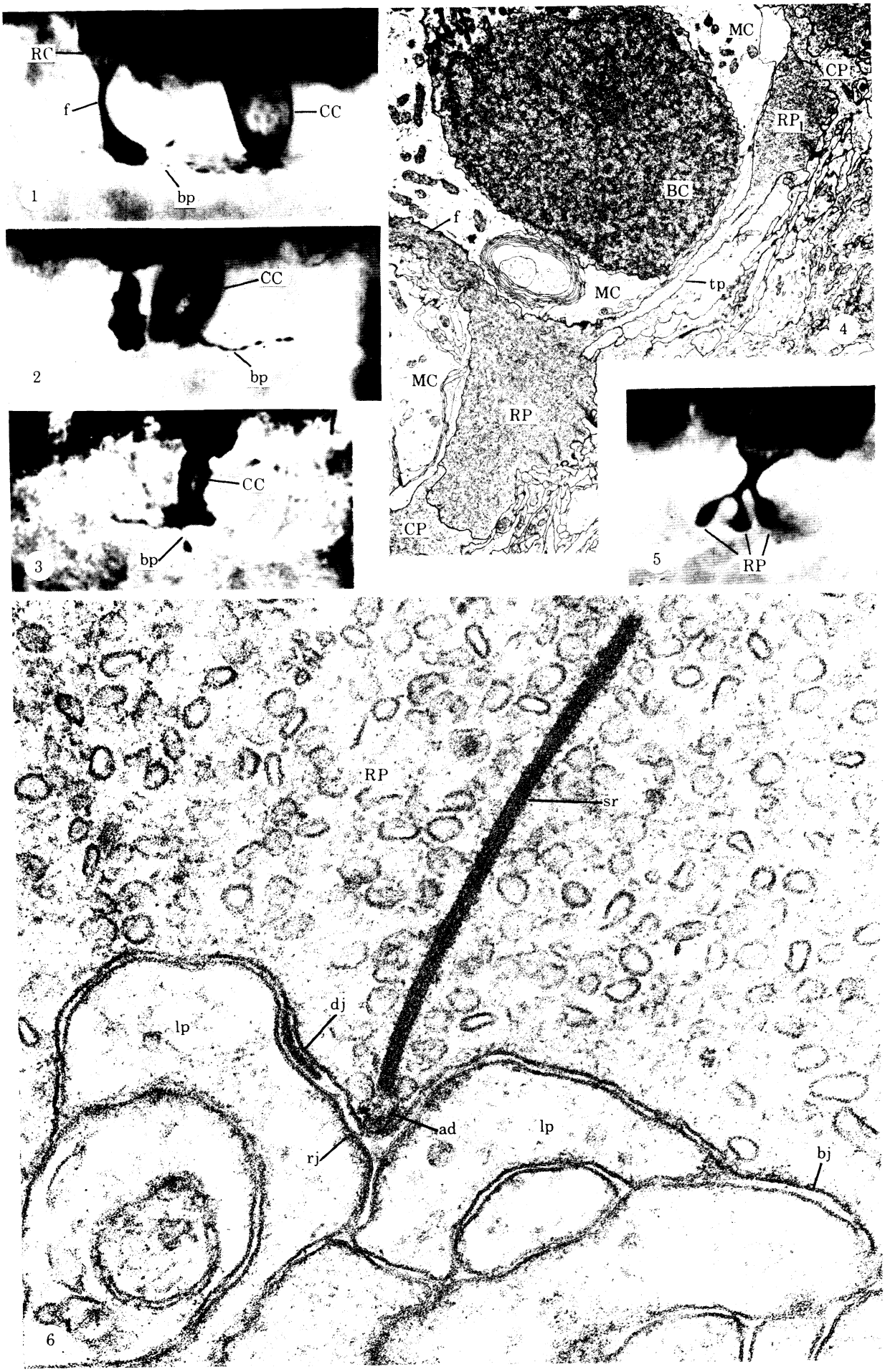
Figure 3. Golgi-stained cone cell (CC). A basal process (bp) extends toward the inner nuclear layer. (Magn. × 800.)

Figure 4. Electron micrograph of the salamander retina. A rod pedicle (RP) gives origin to a tangential process (tp) which ends in an accessory pedicle (RP_1). Both main and accessory pedicles are adjacent to cone pedicles (CP). (*f*), rod cell fibre; (BC), displaced bipolar cell; (MC), Müller cell cytoplasm. (Magn. × 8000.)

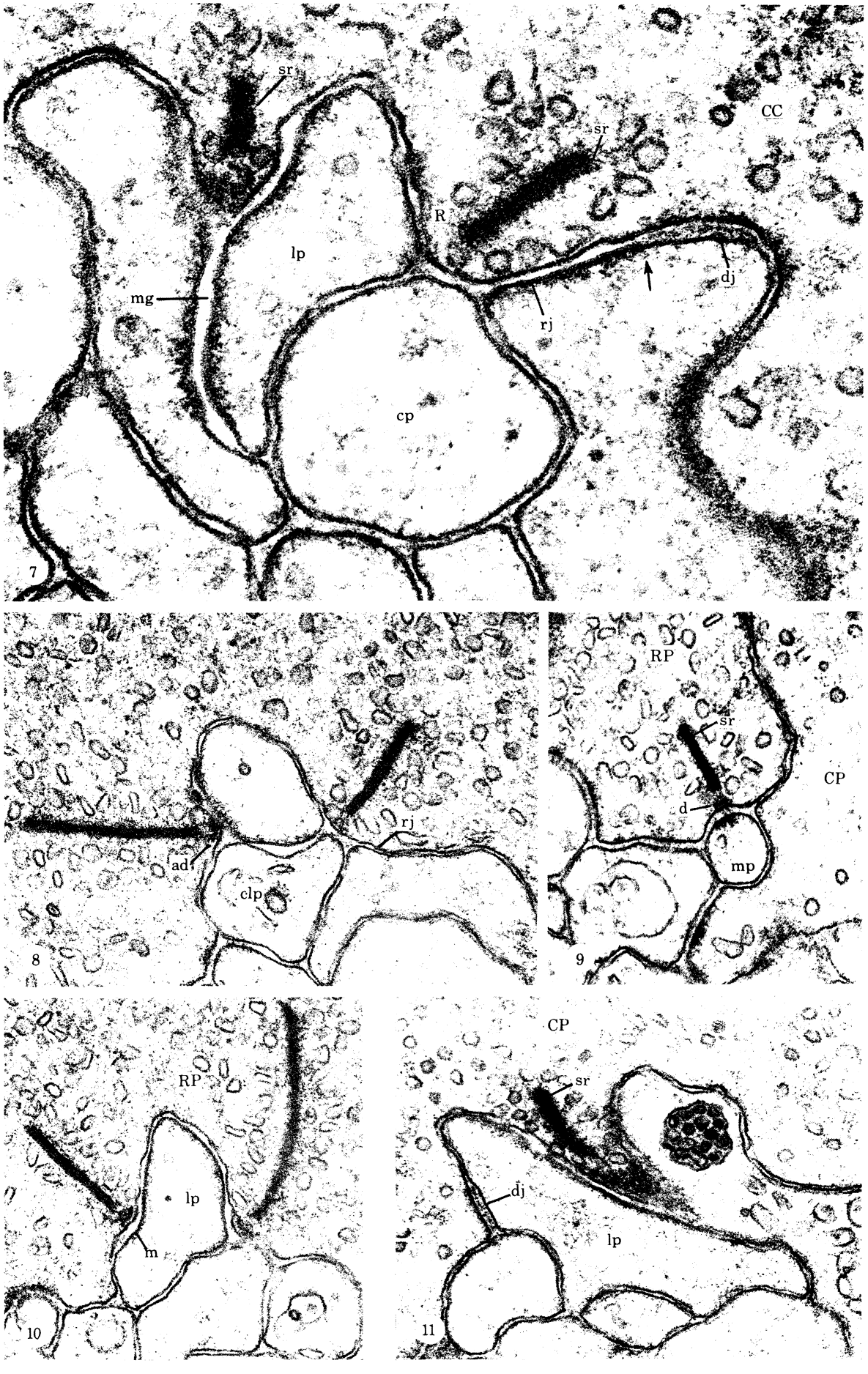
Figure 5. Golgi-stained rod cells showing three pedicles (RP) linked by separate fibres to a thick stem arising from the perikaryon. The outer portions of the cell are covered by silver deposits (see legend figure 1). (Magn. × 800.)

Figure 6. Rod pedicle (RP). At a ribbon junction (rj) the plasma membrane of the lateral processes (lp) is lined by an opaque cytoplasmic layer; both lateral processes, therefore, belong to horizontal cells (see text). A distal junction (dj) of one of the lateral processes with the rod pedicle is defined by a widened intercellular gap bisected by a band of very opaque and homogeneous material; neither of the junctional membranes is lined by opaque cytoplasm. Another process makes a basal junction (bj) of the narrow-gap type; the opaque lining on the inner surface of the rod cell membrane is barely noticeable, but the junction can nevertheless be identified because the intercellular gap is wider than at nonspecialized areas of contact. (sr), synaptic ribbon; (ad), arciform density. (Magn. × 108 000.)

† The term 'junction' implies only an intercellular boundary where the cell surfaces, or the intercellular space, or both, show some kind of structural specialization.



FIGURES 1 TO 6. For legends see facing page.



FIGURES 7 TO 11. For legends see facing page.

make contact with either two or three neuronal processes: those found on either side of the ridges will be referred to in the following as 'lateral processes' (figure 6, plate 47; figure 7, plate 48), while those ending at the apex of the ridges will be termed 'central processes' (figure 7). The resulting images resemble those of the dyads and triads observed in the turtle retina (Lasansky 1971), but in the salamander serial sections showed that the number of processes making contact with a synaptic ridge shows considerable variation; for instance, three or more lateral processes may end at what would seem to be a dyad when examined in a single section. A similar observation has been described in detail in the retina of *Necturus* (Dowling & Werblin 1969).

Lateral processes may show an opaque cytoplasmic lining on the portion of the plasma membrane facing the synaptic ridge and the apposed lateral process (figures 6, 7). This area was previously termed the 'proximal junction' because of its closeness to the apex of the synaptic ridge (Lasansky 1971). The opaque lining is sometimes missing at one or both lateral processes, so that in those instances the only reason to consider the contact a specialized one is the presence of the ribbon on the visual cell side (figure 8, plate 48; figure 31, plate 54). In most cases, when the membrane of both lateral processes shows an opaque lining, the ribbon junctions are more deeply placed in the pedicles than those where the lateral processes lack such lining. Thus, the two kinds of contacts may correspond to the invaginated and superficial ribbon contacts described in *Necturus* (Dowling & Werblin 1969). Nevertheless, exceptions to this correspondence may be found and, furthermore, the inner surface of the pedicles in the salamander retina is often too intricate to decide how deeply a particular process is invaginated, at least in single sections. In contrast, the membrane linings are a more useful identifying feature, since lateral processes showing them belong to horizontal cells (see below).

Serial sections show that central processes do not extend beyond the apex of the synaptic

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Description of plate 48

Figure 7. Two ribbon junctions share a lateral process (lp) at a cone pedicle (CC). Another lateral process makes a distal junction (dj) with the pedicle; note that the opaque cytoplasmic lining on the membrane of the lateral process at the ribbon junction (rj) does not extend (arrow) to the distal junction, which shows instead a similar lining on the cone cell side. The membrane of the lateral processes making the ribbon junction on the left-hand side shows an opaque lining not only on the portion facing the synaptic ridge, but also throughout most of the extent of the medial gap (mg) between the lateral processes. A central process (cp) is close to the apex of the synaptic ridge (R), and does not have an opaque membrane lining at this apical contact. (sr), synaptic ribbon. (Magn. × 126000.)

Figure 8. Rod pedicle. The same process (clp) is central at one ribbon junction and lateral at another. None of the processes making ribbon junctions (rj) shows a membrane lining at the site of the junction (the opaque area on the left-hand side of the uppermost lateral process is probably due to oblique sectioning of the plasma membrane). An arciform density (ad) is seen at only one of the ribbon junctions (Magn. × 65000.)

Figure 9. A rod pedicle (RP) appears to make a ribbon junction with a single process (mp). The junctional gap contains some material, and the plasma membrane of the contacting process is lined by opaque cytoplasm. Near the rod cell membrane there is an accumulation of dense material (d), which is the counterpart of the arciform density observed at other ribbon junctions. (sr), synaptic ribbon; (CP) cone pedicle. (Magn. × 65000.)

Figure 10. A lateral process (lp) of a rod ribbon junction makes an additional ribbon contact with the same pedicle as the single process of a monad (m). (RP), rod pedicle. (Magn. × 65000.)

Figure 11. Distal junction (dj) between a cone pedicle (CP) and a lateral process (lp). The junctional gap is wide and contains a layer of opaque material; the inner surface of the cone cell membrane is lined by a conspicuous opaque layer. (sr), synaptic ribbon (Magn. × 65000.)

ridges (figure 24, plate 52). In turtle cones, the large gap separating both elements made it unclear whether this end contact of the central processes – termed apical junction (Lasansky 1971) – engaged the synaptic ridge or the lateral processes. The uncertainty is removed in the salamander by the close distance (10 to 15 nm) between the tip of the central process and the synaptic ridge (figure 7, plate 48). It seems unnecessary, therefore, to keep referring to this contact as apical junction, and it will be grouped with the one previously termed proximal junction of the lateral processes, under the general label of 'ribbon junction'. This grouping is further justified by the observation that the same process may be central at one ribbon junction and lateral at another (figure 8, plate 48).

Still another type of arrangement is found at rod ribbon junctions. It will be referred to as 'monad' because only one process of a second-order neuron contacts the rod pedicles at such junctions (figures 9, 10, plate 48; figure 18, plate 50; figure 30, plate 53). At some of these junctions the notion that they engage only one process is suggested by the absence of a synaptic ridge, so that the ribbon is approximately perpendicular to the junctional surface. The junctional gap contains some extracellular material, and the plasma membrane of the process making contact with the rod pedicle may or may not show an opaque cytoplasmic lining (figure 9). On the rod cell side there is sometimes an accumulation of opaque cytoplasmic material very near, but not attached to, the plasma membrane (figure 9); this material represents the counterpart of the arciform density (Ladman 1958) observed at other ribbon junctions (figure 6, plate 47). At some other monads the geometry of the contact leaves little doubt that the rod pedicles contact only one process, since other processes appear to be too remote from the ribbon area. Such is the situation when the junctional process invaginates the pedicle (figure 10,) or it is relatively large (figure 30).

Distal junctions of lateral processes

The expression 'distal junction' was used previously to indicate contacts of lateral processes located away from the apex of the synaptic ridge, at the end of the medial gap of cone dyads (Lasansky 1971). The equivalent contacts of salamander cones and rods have a more variable location; thus, they may be adjacent to the ribbon junction (figure 6, plate 47; figure 7, plate 48), or involve a segment of the surface of a lateral process unrelated to the synaptic ridge (figure 11, plate 48). Less than one-fifth of the lateral processes make distal junctions, and all of these show an opaque membrane lining at the ribbon junction (figure 7).

Cone and rod distal junctions differ in appearance. At cones, the distal junctions of lateral processes resemble those found in turtle cones. They consist of a widened intercellular gap containing a rather compact material; the cone cell membrane is lined by a layer of opaque cytoplasm (figures 7 and 11). Instead, at rod distal junctions neither one of the apposed cell membranes is associated with any cytoplasmic densification, and the intercellular material forms a thicker and still more compact plate (figure 6). Similar accumulations of opaque extracellular material have been observed between lateral processes and rod pedicles in the dogfish retina (Stell 1972), and between unidentified receptor pedicles and invaginating processes in the developing retina of bullfrog tadpoles (Nillson & Crescitelli 1969).

Basal junctions

In turtle cones the basal junctions are specialized contacts at the basal surface of the pedicles (Lasansky 1969, 1971). As mentioned above, the corresponding area of the pedicles in the

salamander retina is often difficult to define. Furthermore, processes making basal junctions with the pedicles may be as deeply invaginated into the pedicles as those making ribbon junctions (figure 6, plate 47). Nevertheless, the term 'basal junctions' will still be used on this occasion because of its applicability in a number of other retinas, where such contacts are relatively superficial (e.g. Dowling & Boycott 1966; Dowling 1968).

The major identifying feature of basal junctions is their lack of close association with synaptic ribbons (figures 12 to 17, plate 49). Single sections may sometimes show a ribbon adjacent to a basal junction, but in serial sections it is seen that most of such images represent a boundary line where the basal junction is beginning to disappear, while the ribbon is beginning to enter the picture. In any event, the vast majority of basal junctions are not closely related to ribbons at any point of their expanse (figure 12).

Many fine neuronal processes reaching the pedicles end at basal junctions (figure 12); basal junctions, then, are the only contact between such processes and the photoreceptor cells. A few synaptic vesicles are sometimes seen near the membrane of the pedicles at a basal junction (figure 12), but very often almost none are present in the immediate vicinity of the junctional surface. In fact, it may be said that as a general rule, the band of visual cell cytoplasm adjacent to the surface of the pedicles involved in basal junctions, contains a lower proportion of vesicles than the remainder of the pedicles (figure 13). On the other hand, and with only very infrequent exceptions (figure 15, plate 49), the processes making basal junctions with the pedicles are devoid of vesicles (figure 12).

Basal junctions have the same appearance at cone and rod pedicles, and occur in two slightly different types. At some of them the intercellular gap has a uniform width (about 15 nm) and the photoreceptor cell membrane is lined by an opaque cytoplasmic layer (figure 16), which is frequently rather inconspicuous throughout the extent of the junction (figure 14). At other basal junctions the gap is more irregular and narrower (7 to 13 nm) (figure 6, plate 47; figure 17, plate 49), but it is still wider than at unspecialized intercellular boundaries (5 to 8 nm), so that it provides a criterion for identifying this type of junction even when the opaque lining on the photoreceptor cell membrane is attenuated (figure 6). When present, this lining is usually of irregular thickness (figure 17), but rarely forms knob-like aggregates similar to those found at certain segments of the basal junctions in turtle cones (Lasansky 1971).

The two types of basal junctions in salamander visual cells resemble closely the narrow- and wide-gap segments of the basal junctions in turtle cones (Lasansky 1971); in the salamander, however, the two types are spatially separated. Some processes traced in serial sections make both kinds of basal junctions at different areas of a pedicle; one of those was process lp in figure 31, plate 54, which was seen at other points of the series to make a narrow-gap type of junction, in addition to the wide-gap junction illustrated (figure 31 *g*). More frequently, all the basal junctions of a single neuronal process, or of its branchlets, are of the same type.

A moderately opaque material is found at the basal junctions within the intercellular gap (figures 14, 16 and 17, plate 49). Occasionally, this material shows some periodicity (figure 14), but no special effort was done to establish whether it is organized in a lattice as in turtle cones (Lasansky 1969, 1971). Due to the presence of this extracellular material, in addition to the opaque cytoplasmic lining on the photoreceptor cell membrane, the basal junctions closely resemble the distal junctions of lateral processes at cone pedicles. The two junctions can be distinguished, however, because the intercellular material at cone distal junctions is noticeably more opaque and compact.

An interesting feature of basal junctions is that they can be shared by rod and cone pedicles (figures 13, 14). This arrangement is very frequent for processes reaching the pedicles at the boundary between a rod and a cone.

Interreceptor contacts

Adjacent rod and cone pedicles may make contact at ribbon junctions. At most of those contacts, cone pedicles contribute a lateral process to a rod ribbon junction (figures 18, 19, plate 50). The cone processes are devoid of vesicles, and do not show an opaque lining on the surface membrane facing the synaptic ridge (figures 18, 19). Serial sections showed that such processes may follow a rather long and tortuous path to end at a ribbon junction of an adjacent rod pedicle.

All the cone pedicles serially sectioned through most of their extent (five pedicles) were found to make one ribbon junction – and only one – as lateral processes at an adjacent rod pedicle. A rod pedicle was seen in one instance to contribute a lateral process to a cone ribbon junction (figure 23, plate 51). Ribbon junctions between two adjacent cone pedicles were also observed once; as suggested above, the cone cells involved may have been the members of a double cone. Ribbon contacts between rod pedicles were not observed.

The finding that the pedicles give origin to basal processes which extend horizontally (figure 2, plate 47), suggested that perhaps ribbon contacts also take place between distant visual cells. Only two Golgi-stained cone cells showing single basal processes were suitable for electron microscopy; in both instances the basal processes ended within the plexiform layer without giving any indication about the nature of their end contacts. No attempt was made to trace the basal processes directed towards the inner nuclear layer (figure 3, plate 47), but in agreement with the interpretation of Dowling & Werblin (1969) in the retina of *Necturus*, their end contacts are presumed to be ribbon junctions on the soma of second-order neurons, since

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DESCRIPTION OF PLATE 49

FIGURE 12. Electron micrographs *a* to *e* are from consecutive serial sections. A fine neuronal process (np) is seen approaching a rod pedicle (RP) in *a* and *b*, making a basal junction (bj) in *c*, and ending in *d* and *e*; all that remains of the process in picture *e* is an opaque patch (arrow) due to tangential sectioning of its surface. There is no synaptic ribbon in the immediate vicinity, and only a few vesicles are seen near the rod cell side of the basal junction in micrograph *c*; the neuronal process is devoid of vesicles throughout its thickness. An adjacent cone pedicle (CP) shows a lighter cytoplasmic matrix and smaller synaptic vesicles than the rod pedicle (Magn. × 27 000.)

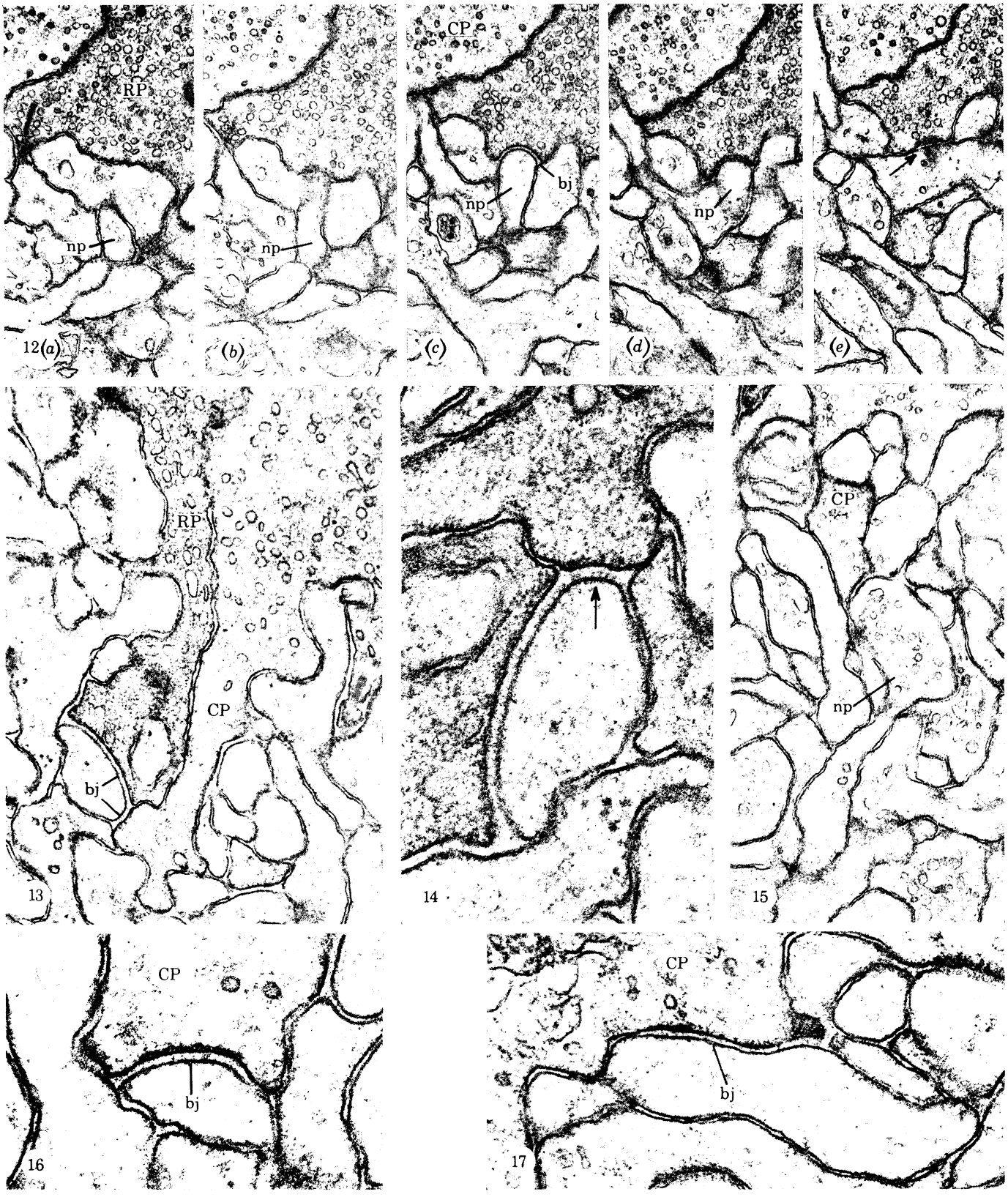
FIGURE 13. A basal junction (bj) is shared by a rod (RP) and a cone (CP). Near the junction the photoreceptor cells are almost devoid of vesicles, in sharp contrast with the abundance of vesicles found elsewhere in the pedicles. (Magn. × 43 000.)

FIGURE 14. Enlargement of a part of figure 13. The basal junction is of the wide-gap type, the gap material shows some suggestion of periodicity (arrow), and the opaque cytoplasmic lining on the plasma membrane of the pedicles is very attenuated. (Magn. × 108 000.)

FIGURE 15. A neuronal process (np) making a basal junction with a cone pedicle (CP) contains numerous vesicles. This type of process is very infrequently found, and it may belong to a special kind of neuron. (Magn. × 32 000.)

FIGURE 16. Basal junction (bj) of the wide-gap type at a cone pedicle (CP). The junctional segment of the cone cell membrane is lined by a thick layer of opaque cytoplasm. (Magn. 86 000.)

FIGURE 17. Basal junction (bj) of the narrow-gap type at a cone pedicle (CP). At the junction, the inner surface of the cone cell membrane shows an opaque lining of uneven thickness. (Magn. × 65 000.)



FIGURES 12 TO 17. For legends see facing page.

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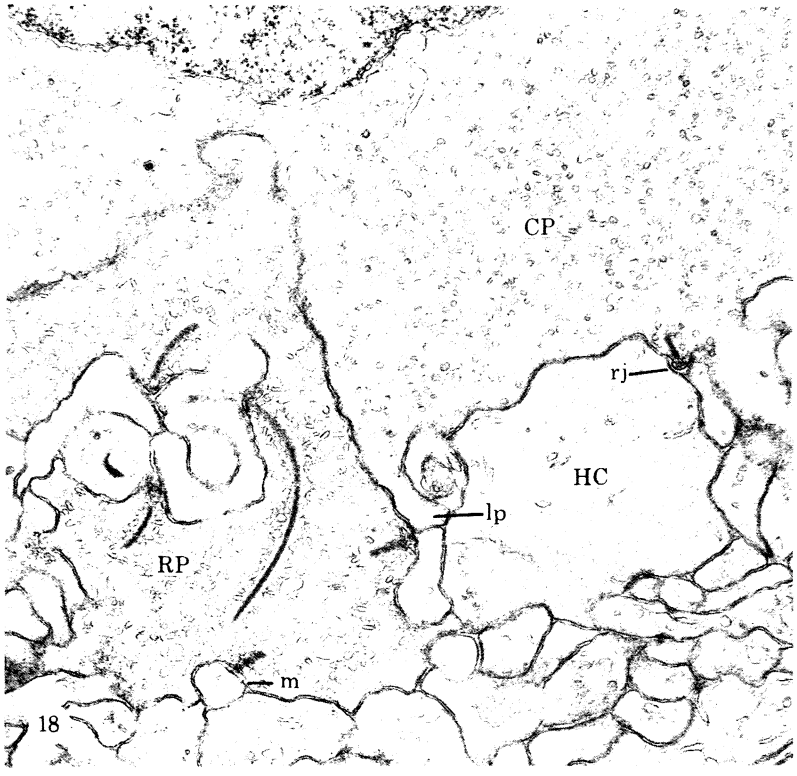

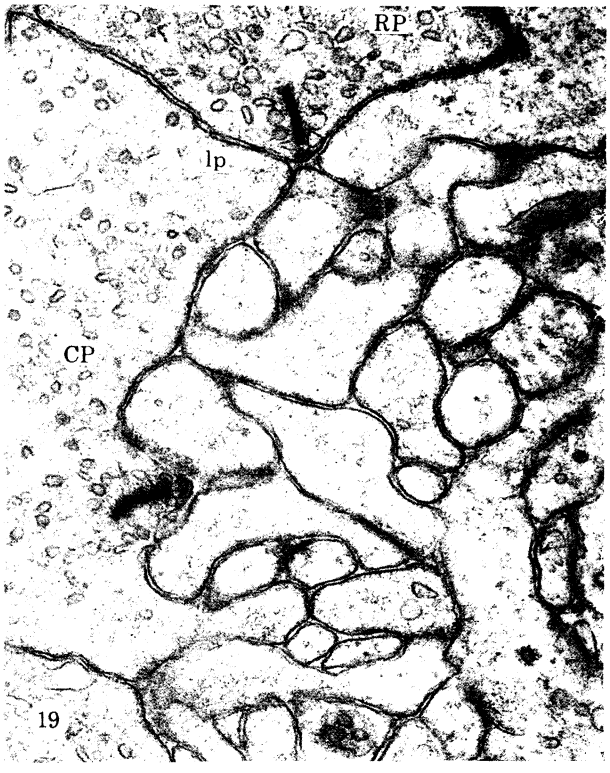

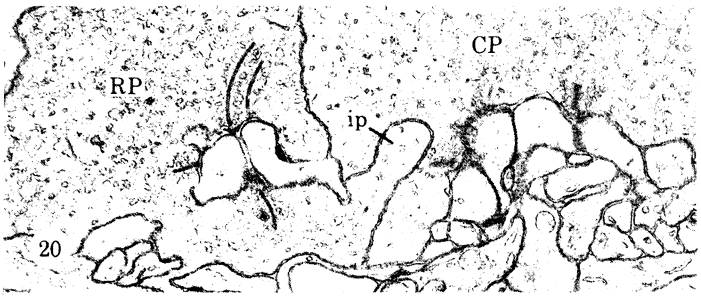

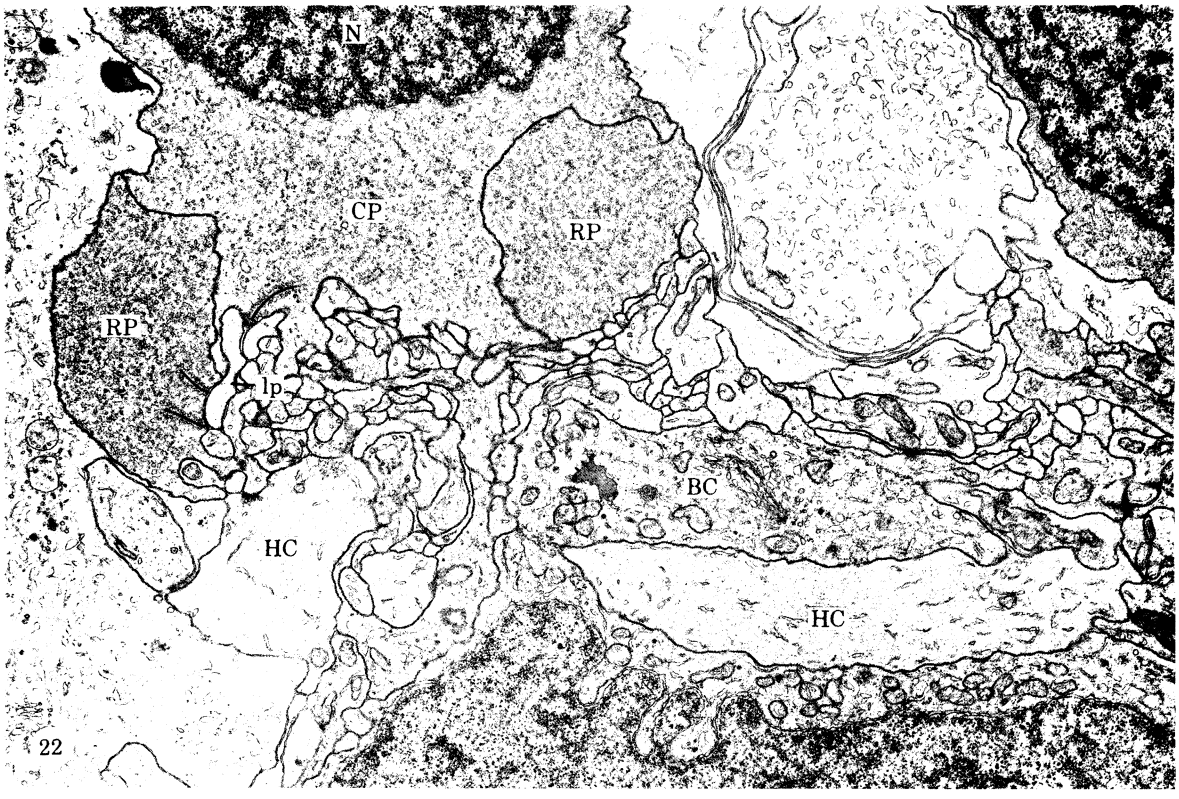

FIGURES 18 TO 22. For legends see facing page.

isolated photoreceptor cell processes making such contacts were sometimes detected in electron micrographs.

Rod pedicles give origin sometimes to finger-like processes which contain only a few vesicles and invaginate the cone pedicles (figure 20, plate 50). When examined in serial sections, these processes are not found to be involved in ribbon junctions, nor do they show any other kind of surface specialization.

Junctions between photoreceptors and horizontal cells

A conspicuous feature of the outer plexiform layer is a stratum of thick tangential processes which contain very few mitochondria, a variable amount of microtubules and smooth endoplasmic reticulum and, predominantly, a filamentous matrix. In low-power electron micrographs, this scarcity of cell organelles results in a characteristically amorphous overall appearance (figure 22, plate 50; figure 23, plate 51). Processes with a similar appearance have been interpreted to belong to horizontal cells in other retinas (Yamada & Ishikawa 1965), and the same identity is assumed for those in the salamander retina, since Golgi-stained horizontal cells show thick processes extending horizontally (figure 21, plate 50). When examined with the electron microscope, such Golgi-stained thick processes were found within the stratum mentioned above, and had the expected scarcity of mitochondria, which are preserved in Golgi preparations (Stell 1965). In contrast, comparably thick tangential portions of bipolar cells are much less numerous and shorter, and light microscopy of Golgi-stained bipolar cells shows that they can only be found at or near the origin of the Landolt clubs. Because of this proximity, those thick bipolar cell segments are easy to identify in serial sections. They never have an 'amorphous' appearance, and contain abundant mitochondria and other cytoplasmic membranes (figure 22, plate 50).

On the other hand, fine branchlets of horizontal cell processes cannot be safely distinguished from bipolar cell dendrites. In serial sections, however, such fine branches can be traced from their origin at the thick horizontal cell processes to their end at the rod and cone pedicles. In agreement with previous findings in other retinas (Stell 1967; Kolb 1970; Lasansky 1971), most

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DESCRIPTION OF PLATE 50

FIGURE 18. A lateral process (lp) of a rod ribbon junction belongs to a cone pedicle (CP); note the absence of vesicles within this process. A monad (m) of the rod pedicle (RP) is also shown. A thick horizontal cell process (HC) makes a cone ribbon junction (rj). (Magn. × 22 000.)

FIGURE 19. One of the lateral processes (lp) of a rod ribbon junction belongs to a cone pedicle (CP), which at this area is almost devoid of synaptic vesicles. None of the three junctional processes shows an opaque lining on the plasma membrane. (RP), rod pedicle. (Magn. × 65 000.)

FIGURE 20. A rod pedicle (RP) extends an invaginating process (ip) containing few synaptic vesicles into a cone pedicle (CP). (Magn. × 13 000.)

FIGURE 21. Horizontal cell stained by the method of Golgi. Thick processes originate from both sides of the cell body; some ascending fine branchlets are also observed (Magn. × 400.)

FIGURE 22. Outer synaptic layer of the salamander retina. This micrograph belongs to a series, so that a bipolar cell process (BC) could be identified by following it to an immediately adjacent Landolt club. The abundant mitochondria and cytoplasmic membranes within the bipolar cell stand in striking contrast to the scarcity of such elements within neighbouring horizontal cell processes (HC). A cone pedicle (CP) flanked by two rod pedicles (RP) is also seen. The cone pedicle can be recognized because the cell nucleus (N) is adjacent to the synaptic area. A single horizontal cell process (lp) makes ribbon junctions with both the cone and one of the rod pedicles. (Magn. × 8 000.)

of those endings were lateral processes of ribbon junctions (figure 23, plate 51). Occasionally, thick horizontal cell processes may be seen as lateral processes, so that the identification of the junctional elements can be accomplished in a single section (figure 18, plate 50; figure 33, plate 56). The remainder of the horizontal cell processes end as central processes (figure 24, plate 52) or as the single processes at monads; such an arrangement has not yet been reported in other retinas. Lateral processes exhibiting an opaque lining on their plasma membrane at the ribbon junction (see above) were always shown in serial sections to belong to horizontal cells. Nevertheless, some lateral processes without such a lining belong also to horizontal cells.

Distal junctions are present only at rod and cone lateral processes that show the opaque cytoplasmic lining at the ribbon junction and, hence, belong to horizontal cells (figures 6, 7 and 23). Some lateral processes contain vesicles (figure 24), but this content is not related to the presence of distal junctions, as established in serial sections. Thus, some lateral processes engaging in distal junctions are devoid of vesicles, while lateral processes with an unusual number of vesicles may not make a distal junction.

Processes from the same horizontal cell may contact both rods and cones. Such a relationship was frequently observed in serial sections, but sometimes a single horizontal cell process makes ribbon junctions with a rod and a cone, and the two contacts may be seen within a single plane of section (figure 22, plate 50; figure 23, plate 51).

Junctions between photoreceptors and bipolar cells

Some of the bipolar cells stained by the method of Golgi during this study are shown in figures 25 to 27, plate 52. As in other submammalian forms, bipolar cell bodies are found at both the inner (figures 25, 26) and outer (displaced bipolars) nuclear layers (figure 27). They all show Landolt clubs, and the dendritic trees may consist of long branchlets ending in a small swelling (figure 25), or of thicker dendrites that split in bouquets of fine endings (figure 26).

Only four Golgi-stained bipolar cells of the type depicted in figure 26 were examined with the electron microscope, and they were all found to make only basal junctions with the rod and cone pedicles. In most instances the structure of the contacts was not clearly discernible, but they were assumed to be basal junctions because the serial sections showed that the stained processes ended at such sites, and no ribbons were found in the immediate vicinity (figure 28, plate 52). At a few contacts, however, the surface specializations associated with basal junctions were more obvious, such as in figure 29, plate 52, which also gives evidence that bipolar cells make basal junctions of the type shared by rod and cone pedicles (figure 14, plate 49).

Description of plate 51

Figure 23. Electron micrographs *a* to *c* represent sections from a series, and their relative order is defined as follows: figure 23*a* is section no. 3; *b*, section no. 1; *c*, section no. 8. Micrograph *a* shows a cone pedicle (CP) – recognized because of the adjacent cell nucleus (N) – flanked by rod pedicles (RP); the fibre (*f*) of one of the rod cells is also seen. The two types of pedicles can also be distinguished because the rods appear characteristically denser. At the outer synaptic layer there is a stratum of horizontal cell processes (HC), which at this magnification appear to have a predominantly amorphous content. One of the horizontal cell processes gives origin to an ascending branch (lp) which makes a rod ribbon junction (rrj), and ends as a lateral process shared by two cone ribbon junctions (crj). The continuity between the thick horizontal cell process and its ascending branch can be better observed in figure 23*b* (arrow). In figure 23*c*, the same horizontal cell ending makes a distal junction (dj) with the cone pedicle and an analogous contact (hj) with another horizontal cell process. (Figures 23*a*, *b*, magn. × 16000; figure 23*c*, × 32000.)

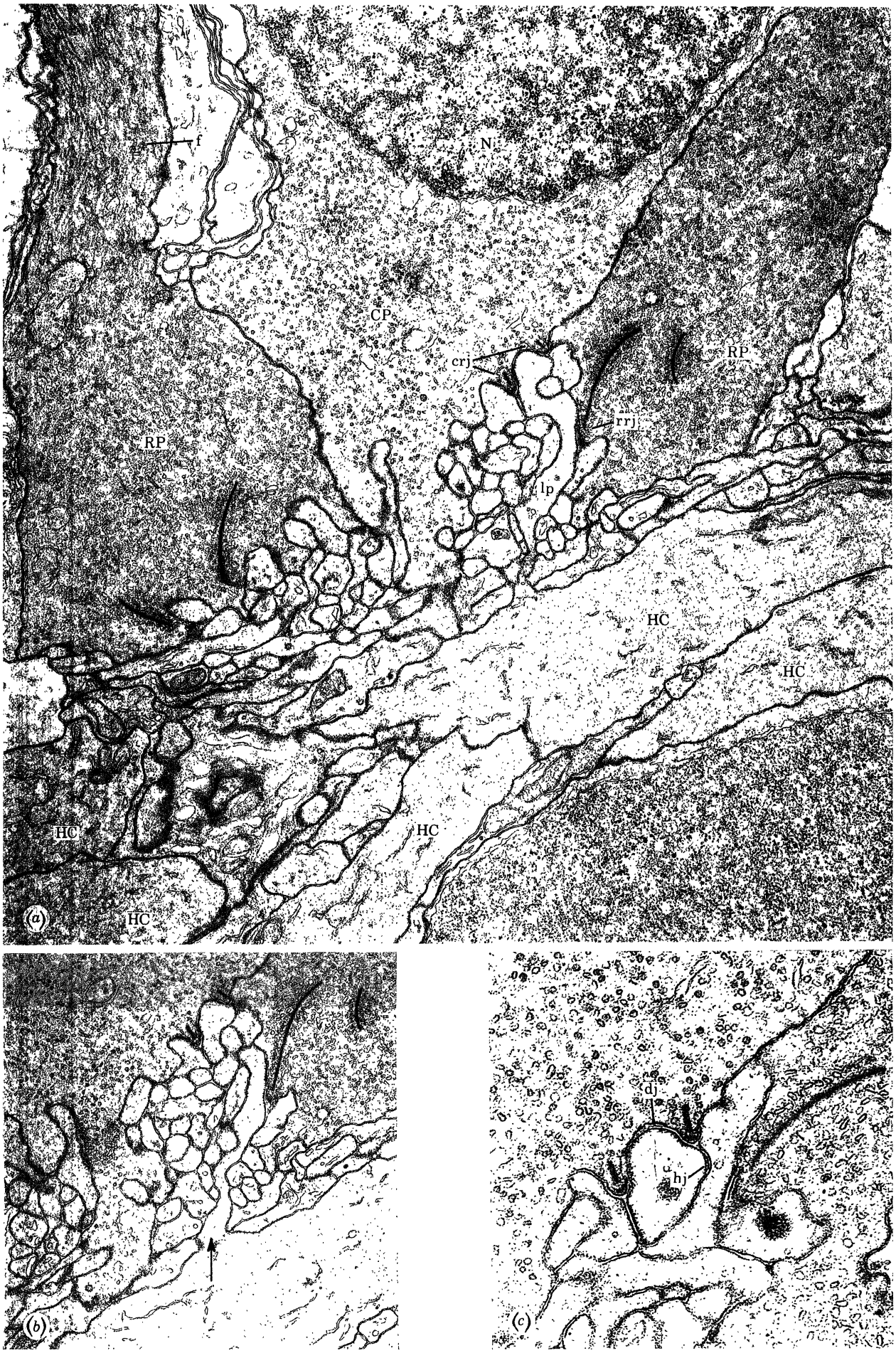

FIGURE 23. For legends see facing page.

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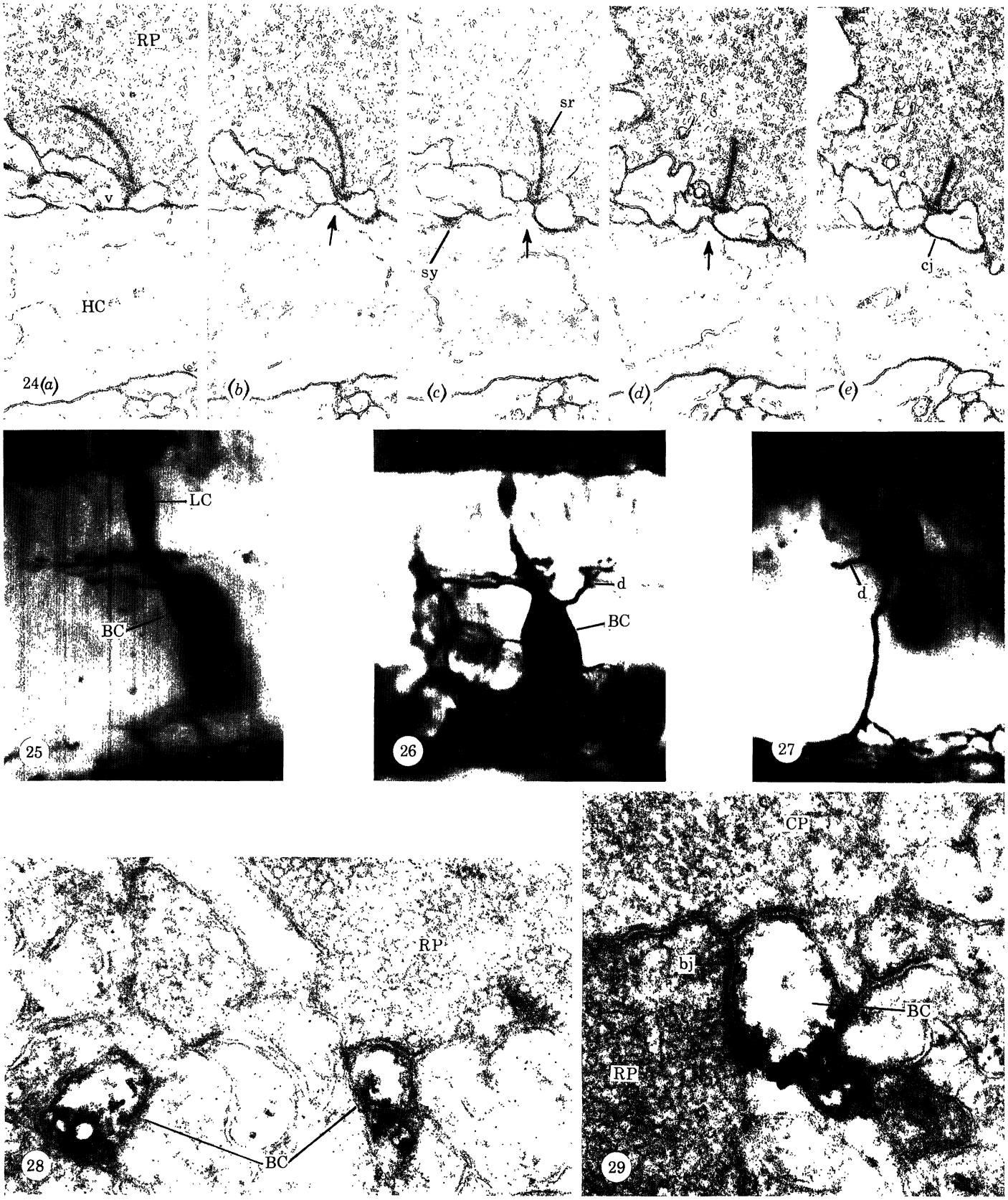

FIGURES 24 TO 29. For legends see facing page.

Basal junctions between bipolar cells and photoreceptor cell pedicles were also observed in serial sections of conventionally fixed material. The field of view was not large enough to trace dendrites to bipolar cell bodies at the inner nuclear layer or to Landolt clubs. Fortunately, displaced bipolars frequently contact pedicles immediately adjacent to their bodies and, furthermore, such cells do not present problems for identification, since they are the only ones having their nuclei at the level of the pedicles (figure 4, plate 47; figure 27, plate 52).

From the study of serial sections it was learned that bipolar cells make also ribbon junctions with the pedicles, either as lateral or central processes or at monads (figure 30, plate 53). Certain processes not traced to their cells of origin, but thought to belong to bipolar cells (see below), make both basal and ribbon junctions with rod or cone pedicles (figure 31, plate 54); sometimes a dendritic branchlet ends at a ribbon junction with one type of pedicle, while another branchlet of the same dendrite ends at a basal junction with another type of pedicle (figure 32, plate 55). Only two or three processes of this kind make contact with each pedicle; this low number has been the main obstacle to their identification.

Junctions between horizontal cells

Areas of close membrane apposition between two horizontal cell processes are found at the outer plexiform layer, or between central and lateral processes of ribbon junctions (figure 24, plate 52), or at the medial gap between apposed lateral processes (figure 33, plate 56). At such contacts the width of the intercellular gap is about 2 nm (figure 34, plate 56).

Another type of specialized contact between two horizontal cells may link both lateral processes at some ribbon junctions, and consists of a widened intercellular gap bisected by a layer of opaque material (figure 35, plate 56). These contacts are entirely similar to rod distal junctions (figure 6, plate 47), and more common between lateral processes making rod ribbon junctions (figure 35), than between those making cone ribbon junctions (figure 23, plate 51).

Description of plate 52

Figure 24. Electron micrographs of serial sections; figures *a* to *e*, correspond to section nos. 1, 3, 5, 7 and 9 respectively. A horizontal cell process (HC) supplies a central process (arrow) to a rod ribbon junction. The illustrated sequence shows that the central process does not extend beyond the apex of the synaptic ridge. In figure 24*a*, a lateral process is seen to contain small vesicles (v). In figures *d* and *e*, the central process shows an area of close membrane apposition (cj) with a lateral process. The horizontal cell is the presynaptic element at a conventional synapse (sy) in figures *b* and *c*. (RP), rod pedicle; (sr), synaptic ribbon. (Magn. × 16000.)

Figure 25. Bipolar cell (BC) stained by the method of Golgi. The fine dendrites extend horizontally and end with small knobs. (LC), Landolt club. (Magn. × 800.)

Figure 26. Golgi-stained bipolar cell (BC). A thick dendrite (d) splits into bouquets of small branchlets. (Magn. × 800.)

Figure 27. Displaced bipolar stained by the method of Golgi. A short dendrite ends with a single knob at an adjacent photoreceptor cell pedicle. (Magn. × 800.)

Figure 28. Electron micrograph of dendritic processes belonging to a Golgi-stained bipolar cell (BC). The process on the right-hand side contacts a rod pedicle (RP), but it is not clear whether the contact is a basal junction. Examination of adjacent serial sections, however, showed that the stained process ended at this site, and no synaptic ribbon was found in the immediate vicinity. (Magn. × 48000.)

Figure 29. Electron micrograph of a Golgi-stained dendritic ending belonging to a bipolar cell (BC). The large empty space within the process results from the loss of most of the silver deposit during sectioning. The stained ending makes a basal junction (bj) with a rod (RP) and a cone (CP). (Magn. × 48000.)

Conventional synapses of horizontal cells

The term 'conventional synapse' will be used here in the same sense intended by Dowling & Werblin (1969), that is, to indicate a specialized contact showing a cluster of vesicles near one of the junctional membranes, such as found at synapses elsewhere (Gray & Guillery 1966).

Contacts of this kind occur within the outer synaptic layer (figure 36, plate 56), and at some of them horizontal cells are easily identified as the presynaptic elements, because they are represented by the typical thick processes with an amorphous content (figure 24, plate 52). At these synapses the intercellular gap is slightly wider, and the inner surface of both membranes is lined by an opaque material that accumulates more conspicuously on the presynaptic side (figure 36). The synaptic vesicles have a diameter of about 30 nm; a few larger vesicles may sometimes be seen, but most of them are of a rather uniform size.

Some of the postsynaptic processes were traced in serial sections to rod or cone pedicles, where they engaged in either basal or ribbon junctions (figure 37, plate 56), and in some instances in both kinds of junctions. It seemed likely, therefore, that they belong to bipolar cells, as postulated for the retina of *Necturus*, also a larval urodele closely related to the salamander (Dowling & Werblin 1969). This presumption received direct confirmation in one occasion, when the displaced bipolar cell of figure 30, plate 53, was found to be postsynaptic at one of these synapses. Bipolar cells are not the only postsynaptic elements, however, as horizontal cells may make synapse also on some as yet unidentified vesicle-containing processes (see below).

Clusters of vesicles of the same size as those at the conventional synapses are frequently seen at unspecialized areas of the horizontal cell surface. The vesicle clusters are related to the cell membrane in the same manner as those at synapses, but serial sections do not show any specialized contact in the vicinity. The apposed elements at such points may be bipolar cells, other horizontal cells, or photoreceptor cell pedicles. When the pedicles are involved, they usually indent the horizontal cell with a small ridge-like projection (figure 38, plate 56).

Conventional synapses involving unidentified elements

Processes containing vesicles 30 to 45 nm in diameter occur within the outer synaptic layer; they have a beaded profile, and the vesicles are seen within the thickened segments. At certain intervals they give origin to ascending branches that invaginate deeply the rod and cone pedicles (figure 39, plate 56), but otherwise do not make any specialized contact with them. These processes could not be traced to any identifiable elements; they simply extended horizontally throughout the longest series available without joining thicker neuronal branches.

DESCRIPTION OF PLATE 53

FIGURE 30. Consecutive serial sections illustrating a displaced bipolar cell making two ribbon junctions with a rod pedicle. The displaced bipolar (BC) in figure 30*a* can be identified because these cells are the only ones having their nuclei (N) at the same level as the photoreceptor cell pedicles (the orientation of the outer synaptic layer in this picture is indicated by the direction of the thick dendrite originating from the bipolar cell). In figures 30*b* to *d*, the bipolar cell dendrite (D) makes a monad (m) with a process (rp) that can be followed to figure 30*h*, where it is found to join a rod pedicle (RP). Starting back from figure 30*h*, the thick bipolar cell dendrite gives origin in figure 30*g* to a fine branchlet (db) which can then be traced through figures 30*f–d*, to its ending as a lateral process at a rod ribbon junction in figure 30*c* (the other lateral process belongs to a horizontal cell). In figure 30*b*, all that remains of the dendritic branchlet is an opaque patch (arrow) due to tangential sectioning of its surface. (Magn. × 21 000.)

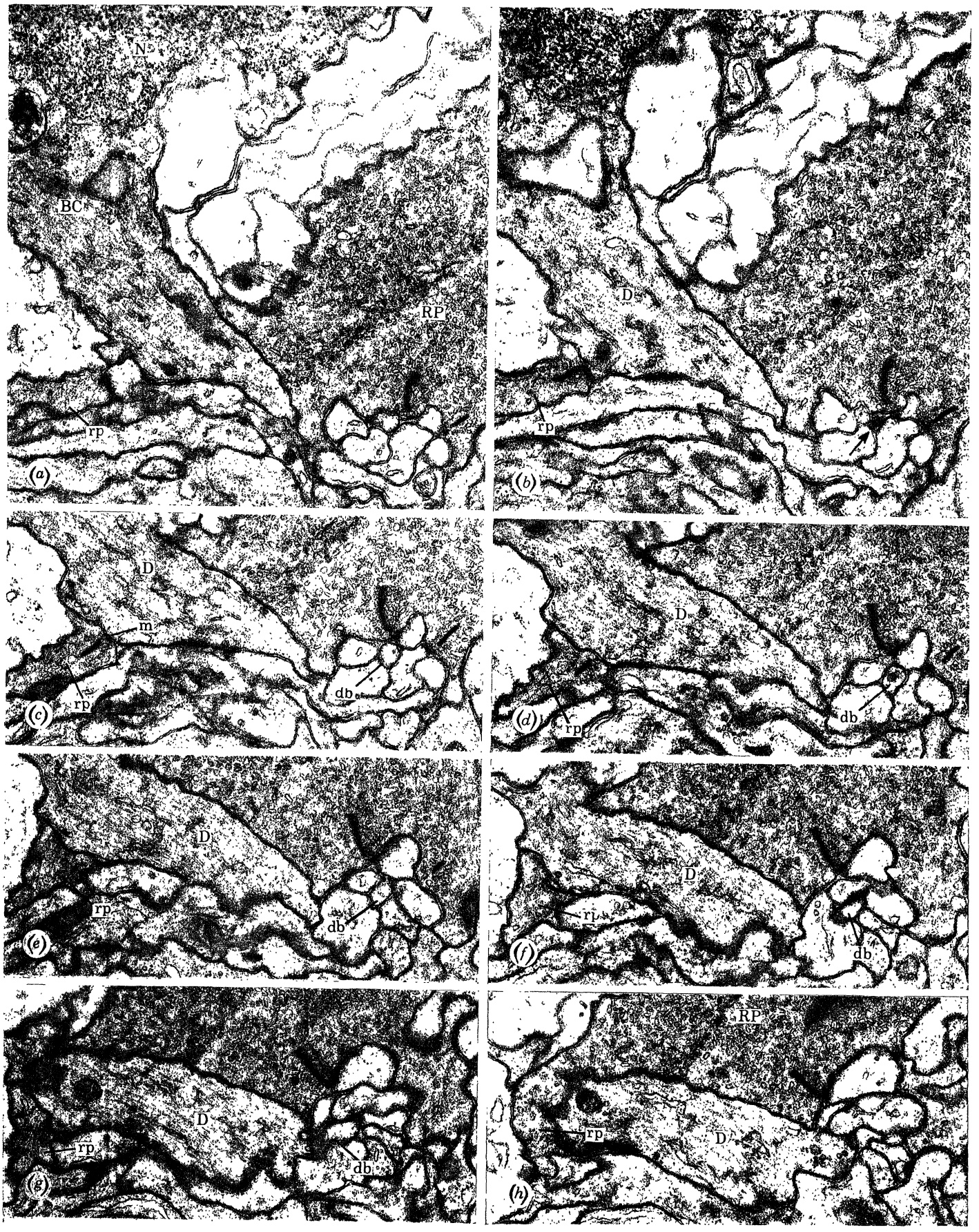

FIGURE 30. For legends see facing page.

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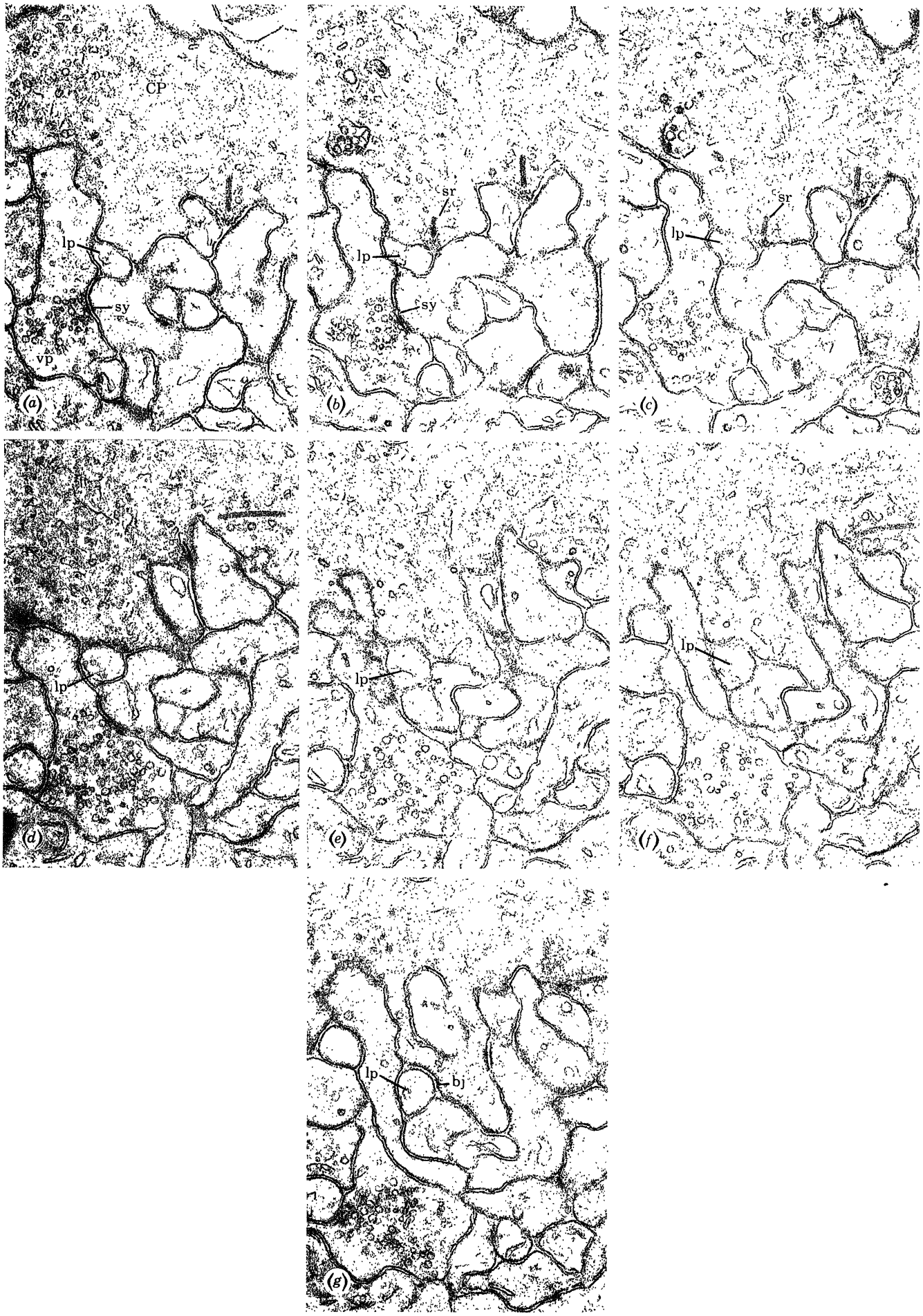

FIGURE 31. For legends see facing page.

The vesicle-containing processes are postsynaptic to horizontal cells at the conventional synapses described above. They make in turn conventional synapses (figure 31, plate 54; figures 40, 41; plate 56) on neuronal processes which are engaged in basal and/or ribbon junctions with the pedicles (figure 31), and are thought to belong to bipolar cells. The two kinds of conventional synapses are similar in appearance; nevertheless, since the vesicle-containing processes have vesicles with a larger and non-uniform diameter, their synapses can be distinguished from those at which horizontal cells are the presynaptic side (compare figures 36 and 41, plate 56). In one instance, a presynaptic process containing the larger type of vesicles was traced to an identifiable horizontal cell process, but could not be traced to the pedicles to find out whether it gave origin to the typical invaginating branches. This is the only hint available on the nature of the vesicle-containing processes, and it suggests that they may be part of a second type of horizontal cells or – more unlikely because of the differences in vesicle size – a second kind of process from a single horizontal cell type.

Discussion

Figure 42 summarizes the present findings. The specialized contacts of rod and cone pedicles in the retina of the salamander are similar in variety and nature to those previously reported in turtle cones (Lasansky 1971), while the neuronal relations at the outer synaptic layer appear to be somewhat more complex than previous reports in other retinas have suggested.

Horizontal and bipolar cells engage in two kinds of contacts with the pedicles (figure 43). Identified bipolar cells made either ribbon or basal junctions, but some unidentified processes which are likely to belong to bipolar cells were seen in serial sections to make both kinds of junctions with the same and/or different pedicles. Such processes are thought to originate from bipolar cells, because horizontal cells, which also engage in ribbon junctions, were not found to make basal junctions. Central processes at ribbon junctions in the monkey retina, shown by Kolb (1970) to belong to rod and invaginating midget cone bipolars, as well as central processes of cone triads in the turtle retina, have recently been reported (Lasansky 1972) to make two kinds of contacts with a single photoreceptor cell ending. One of them, previously termed apical junction, (Lasansky 1971) has been above homologized to a ribbon junction; the other, termed distal junction of the central processes, has been suggested before to be identical to a basal junction (Lasansky 1971, 1972) because of its lack of close association with a ribbon and the presence of an intercellular material similar in opacity and periodic organization to that found

Description of plate 54

Figure 31. Serial sections of a neuronal process making a basal junction and a ribbon junction with the same cone pedicle (CP). Some micrographs have been omitted from the series, and the relative order of those shown is as follows: Figure 31*a* is no. 1; *b*, no. 3; *c*, no. 4; *d*, no. 6; *e*, no. 7; *f*, no. 8; *g*, no. 10. Process lp is a lateral process at a cone ribbon junction in figures 31*b* and *c* (neither lateral process has an opaque membrane lining), and makes a basal junction of the wide-gap type (bj) with the same pedicle in figure 31*g*. The basal junction can be distinguished from a cone distal junction of a horizontal cell, because distal junctions show clearly the presence of opaque material within the gap (see figure 23*c*, plate 51, for comparison at the same magnification). The other lateral process of the same ribbon junction, also found in other micrographs of the series to make a basal junction with the cone pedicle, is postsynaptic (sy) to a vesicle-containing process (vp) in figures 31*a*, *b*. This synapse can be distinguished from those at which thick horizontal cell processes are presynaptic because of differences in vesicle size (see text); the vesicle-containing process invaginates the cone pedicle, without making any junctions, at other points of the series (not shown). (sr), synaptic ribbon. (Magn. × 32,000.)

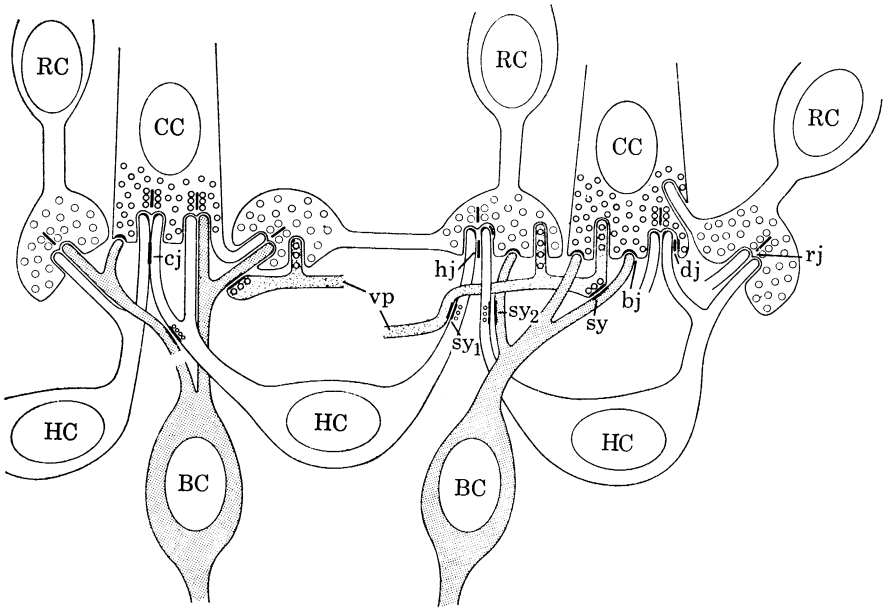

FIGURE 42. Diagram of the outer synaptic layer in the retina of the salamander. Cone pedicles are continuous with the perikaryon (CC), while rod pedicles are linked to the perikaryon (RC) by means of a fibre. One of the rod cells illustrated has an accessory pedicle at the end of a tangential process arising from the main pedicle. Rod pedicles make ribbon junctions on cone cell processes; a cone was also found in one instance (figure 23) to make a ribbon junction on a rod (not represented). Rod cell processes invaginate the cone pedicles without otherwise making any specialized contact. Horizontal cells (HC) engage in ribbon (rj) and distal (dj) junctions with the photoreceptor cell pedicles. A single horizontal cell may contact both rods and cones. Two types of bipolar cells (BC) are represented: one makes only basal junctions (bj) with rod and cone pedicles; the other makes ribbon and probably also basal junctions. Since processes ending at both basal and ribbon junctions could not be traced to their cell bodies, their origin from bipolar cells is hypothetical, as indicated by a break in the diagram. A single bipolar cell may contact both rods and cones. Unidentified vesicle-containing processes (vp) invaginate deeply the pedicles without engaging in any junctions with them, and synapse (sy) on presumed bipolar cell dendrites. The vesicle-containing processes are in turn post-synaptic to horizontal cells (sy_1) which synapse also on bipolar cell dendrites (sy_2). Horizontal cells are linked by two kinds of junctions: close membrane appositions (cj) and contacts showing a widened gap occupied by a layer of opaque material (hj).

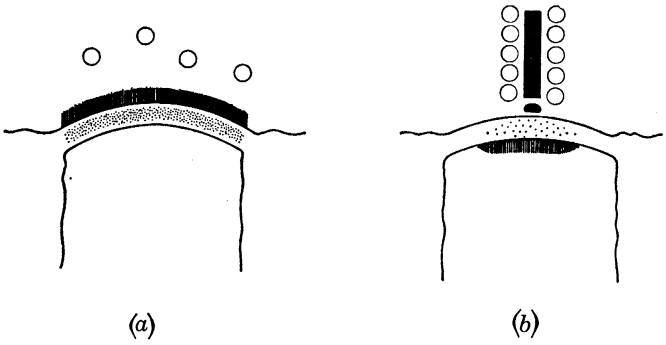

FIGURE 43. Diagrammatic representation of the two types of contacts between photoreceptor cells and second-order neurons. The common features of basal junctions and distal junctions of lateral processes, summarized in diagram (*a*), consist of lack of association with a ribbon, presence of opaque material within the intercellular gap, and an opaque lining on the inner surface of the photoreceptor cell membrane (not seen at rod distal junctions). At ribbon junctions (*b*), the opaque membrane lining, when present, is found on the inner surface of the membrane of the second-order neuron.

at basal junctions. It seems possible, therefore, that the processes making basal and ribbon junctions in the salamander retina originate from bipolar cells that belong to the same group as the invaginating bipolars found elsewhere.

A second type of bipolar cell occurring in other retinas is involved in only basal junctions – known also as superficial or flat contacts (Kolb 1970) – with the photoreceptor cell endings (Kolb, Boycott & Dowling 1969; Kolb 1970; Lasansky 1971, 1972). Examples of this type of cells are provided by the flat bipolars (diffuse or midget) of the monkey retina (Kolb *et al.* 1969; Kolb 1970). The Golgi-stained bipolar cells of the salamander examined with the electron microscope may belong to this group, since they were found to make only basal junctions. Nevertheless, this interpretation can only be regarded as tentative, since not enough cells were examined to exclude the possibility that additional processes making ribbon junctions were not stained due to incomplete impregnation.

Ribbon or basal junctions may be the only contacts between the pedicles and single dendritic branches; it seems safe to assume, therefore, that both kinds of junctions are synapses. Since they differ in structure (figure, 43) it would be reasonable to expect that they are endowed with different functional properties as well. It is generally believed that at the ribbon junctions the photoreceptor cells are the presynaptic elements, and recently Gray & Pease (1971) have pointed out the similarities between the structures found at chemical presynapses and those associated with the ribbons, as well as between the so-called postsynaptic thickening, or subsynaptic web (DeRobertis 1967), and the opaque lining on the membrane of the lateral processes. On the other hand, the basal junctions offer no obvious clues to their possible synaptic mechanism or polarity. Their structure shows no common aspects with the findings at electrical junctions, which are characterized by a close membrane apposition (Brightman & Reese 1969), while the width and organized opaque content of the junctional gap are consistent with what is observed at some chemical synapses (Van der Loos 1964; Gray & Guillery 1966; DeRobertis 1967). Nevertheless, the main criterion to establish the nature and polarity of a chemical synapse – namely the presence of a cluster of vesicles near the presynaptic membrane – is missing at either side of the basal junctions. If the opaque cytoplasmic lining on the receptor cell membrane at the basal junctions is interpreted to be the equivalent of a subsynaptic web, it would be implied that the photoreceptors are the postsynaptic elements, but then most of the processes making basal junctions with the pedicles are devoid of vesicles throughout their extent. Finally, while abundant vesicles are found within the photoreceptor cell pedicles, they could conceivably be related only to the ribbon junctions, since they are fewer near the basal junctions. Thus, it is not possible at this time to decide on a structural basis alone, which is the direction of synaptic transmission at the basal junctions. The answer to this question is needed to understand, for instance, whether those bipolar cells that engage in both basal and ribbon junctions receive a dual input from the receptors or feed back on them.

The junctions of horizontal cells present additional problems of interpretation. The architecture gives in this case no basis to assume that the distal junctions of the lateral processes are synapses: since they are always located near the ribbon junctions, it cannot be reasoned – as it can for basal and ribbon junctions – that the only purpose of any horizontal cell process reaching the pedicles is to establish contact at a distal junction. Cone distal junctions, however, differ in appearance from junctions thought to have only a mechanical role, such as zonulae or maculae adhaerentes (Farquhar & Palade 1963) because of the asymmetric disposition of the opaque cytoplasmic material associated with the junctional areas, an opaque lining being

found only on the cone cell membrane. Instead, it seems possible that cone distal junctions are synapses because of their structural resemblance to basal junctions, but the same problems as with the latter arise in trying to establish their polarity. Since electrophysiological studies on the turtle retina have shown the existence of a horizontal cell feed-back on cones (Baylor, Fuortes & O'Bryan 1971), it was previously proposed that the ribbon and distal junctions between horizontal cells and cones could be oppositely polarized synapses (Lasansky 1971). The present observations on serial sections make this interpretation difficult, since most lateral processes engaged in distal junctions contain few or no vesicles. Again, the findings emphasize the similarities between cone distal junctions and basal junctions, and suggest that the two types of contacts have the same functional role. As an extension of this view, it may be speculated that the dual contacts of horizontal cells with cones are functionally equivalent to the dual contacts attributed to some bipolar cells.

No similar argument could be developed regarding the dual contacts between rod pedicles and horizontal cells, since rod distal junctions differ markedly in structural detail from basal junctions. In fact, rod distal junctions do not even remotely resemble anything known elsewhere to be a synapse, nor are they comparable to desmosomal structures because the latter exhibit a densification of the cytoplasm underlying both junctional membranes (Farquhar & Palade 1963). Thus, it cannot be stated whether the rod distal junctions represent attachment plates, nor whether they may have a role in cellular interaction. Any discussion on these matters must be postponed until electrophysiological work establishes the nature of the interactions between rods and horizontal cells in the salamander retina, and until such interactions can be compared with those between cones and horizontal cells.

Other interesting areas of contact between photoreceptors and horizontal cells are represented by the occasional finding of lateral (horizontal cell) processes containing abundant vesicles, and by the vesicle clusters at unspecialized sites of the horizontal cell surface. Whether these structural arrangements have a functional role in cell interaction cannot be speculated upon. A different view may be expressed with regard to the vesicle-containing processes that invaginate the pedicles, as it would seem likely that the purpose of such a relationship is to bring about some kind of interaction. In any event, all these contacts may be given consideration as alternative or additional sites of horizontal cell feedback on the receptors since, as discussed above, it is uncertain whether the distal junctions of the lateral processes are involved in such feedback.

The vesicle-containing processes remained unidentified, although a single observation

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Description of plate 55

Figure 32. The two branches of a neuronal process end at different pedicles; one at a basal junction with a cone, the other at a ribbon junction with a rod. Some micrographs have been omitted from the series when not essential to follow the processes; the relative order of those shown is as follows: figure 32*a* is no. 1; *b*, no. 3; *c*, no. 5; *d*, no. 7; *e*, no. 8; *f*, no. 9; *g*, no. 10; *h*, no. 11; *i*, no. 12; *j*, no. 13; *k*, no. 15; *l*, no. 17. In figure 32*a* a dendritic branchlet (db) makes a basal junction (bj) with a cone pedicle (CP); the junctional nature of the contact is indicated by the increased width of the intercellular space, the parallelism of the cell membranes, and the opaque lining on the inner surface of the cone cell membrane. A neuronal process (np) which db joins later on is also seen in figure 32*a*; both processes can be followed in figures 32*b–f*, and then db joins np in figures 32*g* and *h* (db+np). The resulting process (db_1) can be followed in subsequent micrographs to a ribbon junction (rj) with a rod pedicle (RP) in figure 32*l*. It should be emphasized that both the basal and ribbon junctions are terminal contacts, since both db and db_1 ended a few sections beyond those illustrated without making any other contact. (Magn. × 21000.)

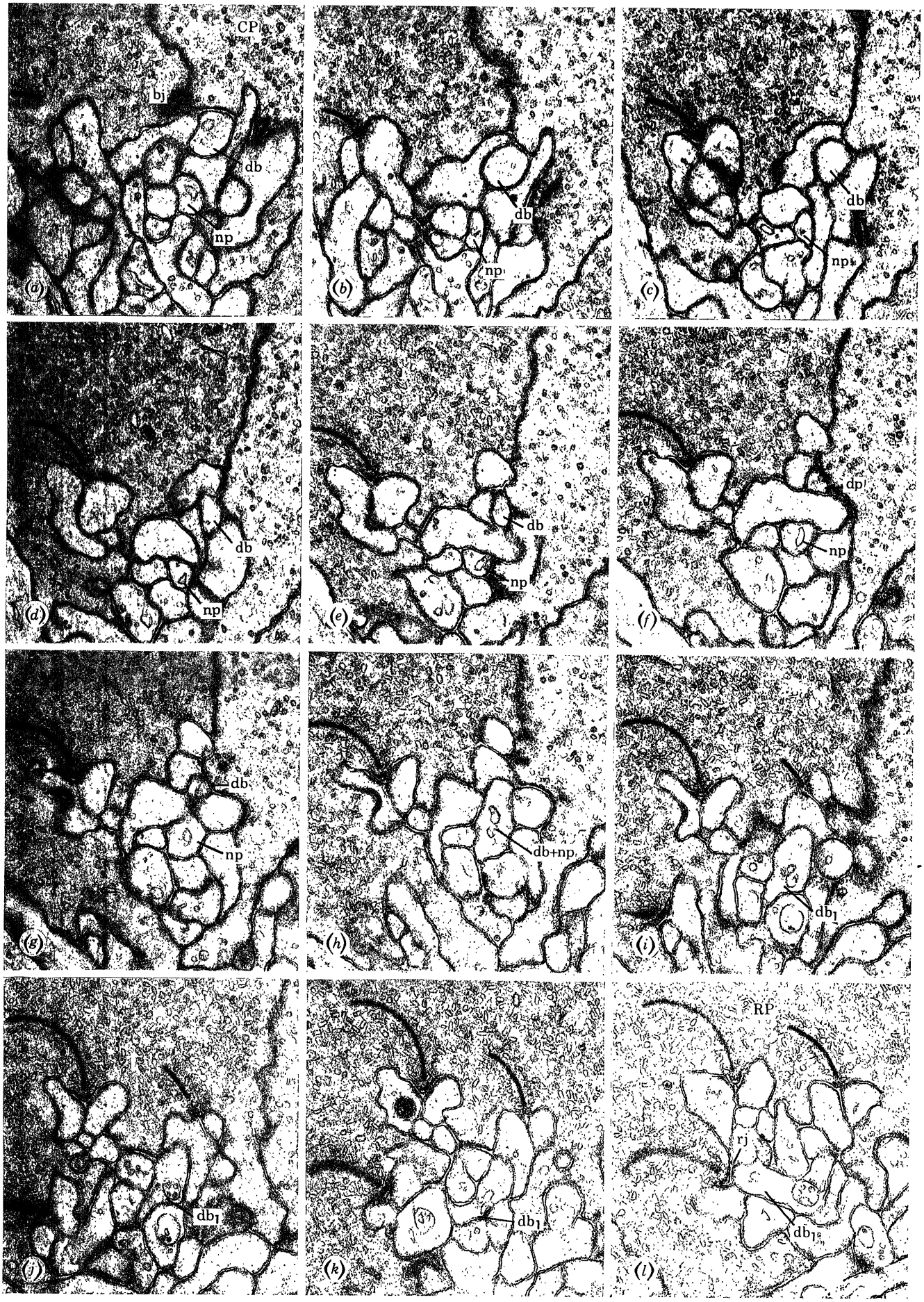

FIGURE 32. For legends see facing page.

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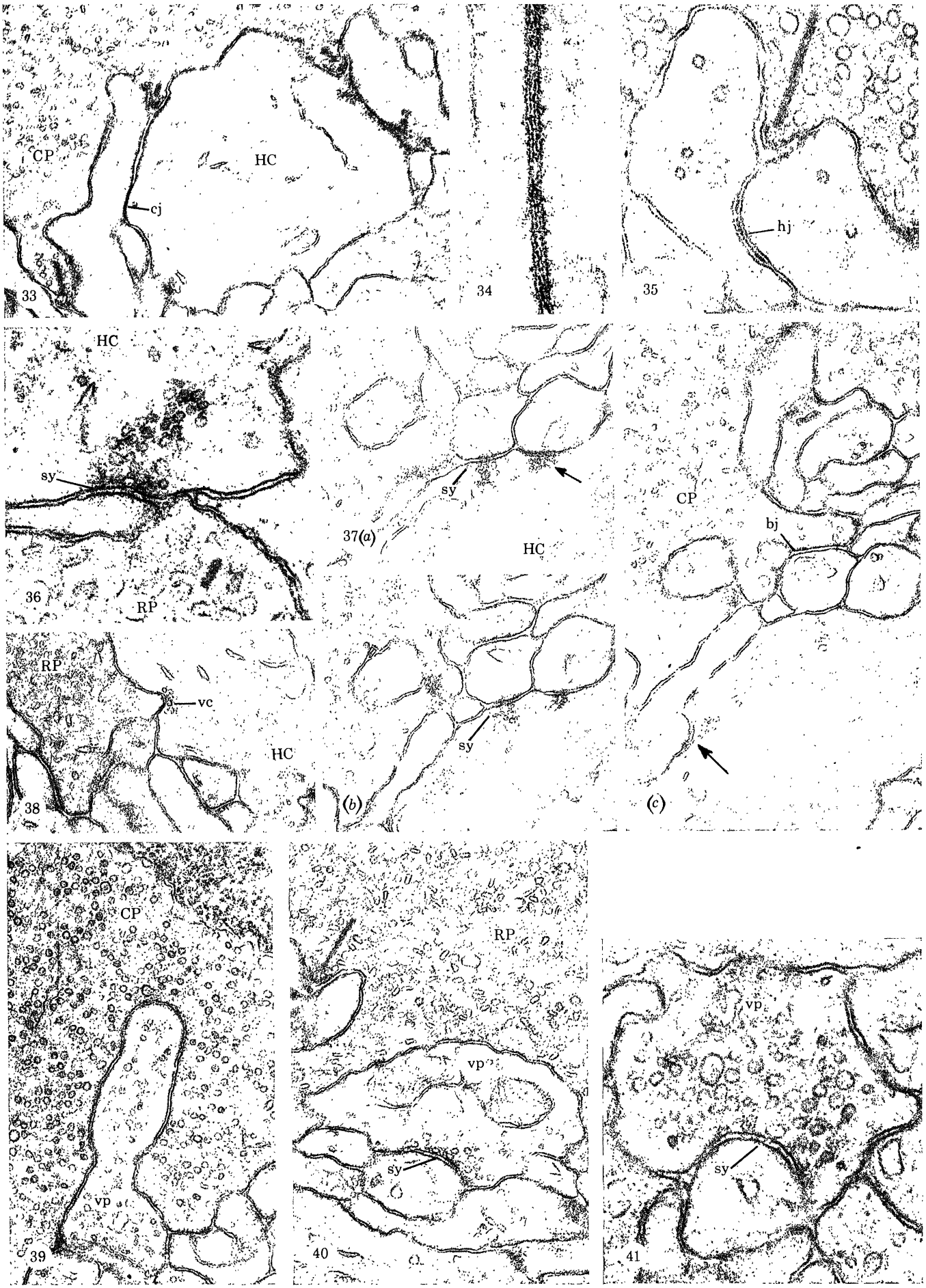

FIGURES 33 TO 41. For legends see facing page.

suggested that they may belong to horizontal cells. Whether or not they indicate the existence of a second horizontal cell type, they seem to provide, on the basis of synaptic vesicle size, a second type of synapses on bipolar cells, in addition to those where identifiable horizontal cells are presynaptic elements. Since horizontal cells are presynaptic also to the vesicle-containing processes, at least two indirect synaptic pathways may be described between photoreceptors and bipolar cells: (*a*) photoreceptor → horizontal cell (process with amorphous content) → bipolar cell, as already postulated in *Necturus* (Dowling & Werblin 1959); (*b*) photoreceptor → horizontal cell → vesicle-containing process → bipolar cell. A third pathway, photoreceptor → vesicle-containing process of a horizontal cell → bipolar cell, would result if the vesicle-containing processes belong in fact to horizontal cells. Lateral actions of distant receptors on bipolar cells in the retina of *Necturus* have been attributed to horizontal cell synapses on bipolar cell dendrites (Dowling & Werblin 1969). The present observations on the salamander retina support such a model to the extent that horizontal cells also have been found to make synapses on bipolar cells. Nevertheless, the finding of additional pathways to mediate peripheral effects on bipolar cells suggests that lateral interactions at the outer plexiform layer of the salamander retina are of a more complex nature than those resulting only in a centre-surround antagonism.

Two types of specialized contacts between horizontal cells were found. Areas of close membrane apposition have been already reported in other retinas (Yamada & Ishikawa 1965; Witkovsky & Dowling 1971; Lasansky 1972); those in the salamander retina are very similar to the 'gap' junctions observed in other nervous tissues (Brightman & Reese 1969), and may also mediate electrical coupling between horizontal cells. Evidence for such coupling has already been presented in the retina of the dogfish (Kaneko 1971). The second type of junction between

Description of plate 56

Figure 33. Close membrane apposition (cj) between two lateral processes of a cone ribbon junction. One of the lateral processes (HC) has the typical appearance that identifies it as belonging to a horizontal cell. The other lateral process was also traced to a horizontal cell in other sections of the series to which this picture belongs. (CP), cone pedicle. (Magn. × 32000.)

Figure 34. Close membrane apposition between two horizontal cell processes. (Magn. × 210000.)

Figure 35. Specialized contact (hj) between the lateral processes of a rod ribbon junction. Both lateral processes belong to horizontal cells, and the contact consists of a widened intercellular gap occupied by a layer of homogeneous material. (Magn. 54000.)

Figure 36. Conventional type of synapse (sy). A horizontal cell process (HC) is the presynaptic element. RP, rod pedicle. (Magn. × 65000.)

Figure 37. Consecutive serial sections. A horizontal cell (HC) is presynaptic (sy) in figures 37*a*, *b* to a process that makes a basal junction (bj) with a cone pedicle (CP) in figure 37*c*. The same horizontal cell makes more conventional synapses on other neuronal processes (arrows). (× Magn. 32000.)

Figure 38. A cluster of vesicles (vc) is found near the plasma membrane of a horizontal cell (HC) at a point where the cell surface is indented by a rod pedicle (RP). No surface specialization is associated with the vesicle cluster in this or adjacent sections. (Magn. × 32000.)

Figure 39. A cone pedicle (CP) is deeply invaginated by a vesicle-containing process (vp). (Magn. × 32000.)

Figure 40. A vesicle-containing process (vp) invaginating a rod pedicle (RP), is presynaptic (sy) to another neuronal process. In other micrographs of the series to which this figure belongs, the postsynaptic process was found to make ribbon and basal junctions with the rod pedicle. (Magn. × 32000.)

Figure 41. A vesicle-containing process (vp) is the presynaptic element at a conventional synapse (sy). The synaptic vesicles have a variable diameter, but most of them are larger than those in figure 36. In an adjacent section, the vesicle-containing process was seen to invaginate a cone pedicle. (Magn. × 65000.)

horizontal cells is represented by the widened intercellular gaps containing a layer of opaque material; these junctions appear to be identical to rod distal junctions, and their significance is equally obscure. Again, a discussion of this problem requires electrophysiological data on the interactions between horizontal cells in the salamander retina.

The specialized contacts between receptor cell pedicles in the retina of the salamander consist of ribbon junctions, and of rod processes invaginating the cones. While little can be said about the latter, the ribbon junctions suggest the possibility of synaptic interaction between rods and cones. Cone basal processes have been found in the turtle retina to end at the dyads of other cone pedicles (Lasansky 1971), but not as close to the synaptic ridges as photoreceptor cell processes in the salamander retina; therefore, no clear analogies can be drawn at present between the two findings. In the turtle retina, electrophysiological evidence of cone cell interaction has been already obtained (Baylor *et al.* 1971). The application of similar experimental methods to the salamander retina may help to clarify the significance of the ribbon junctions between rods and cones.

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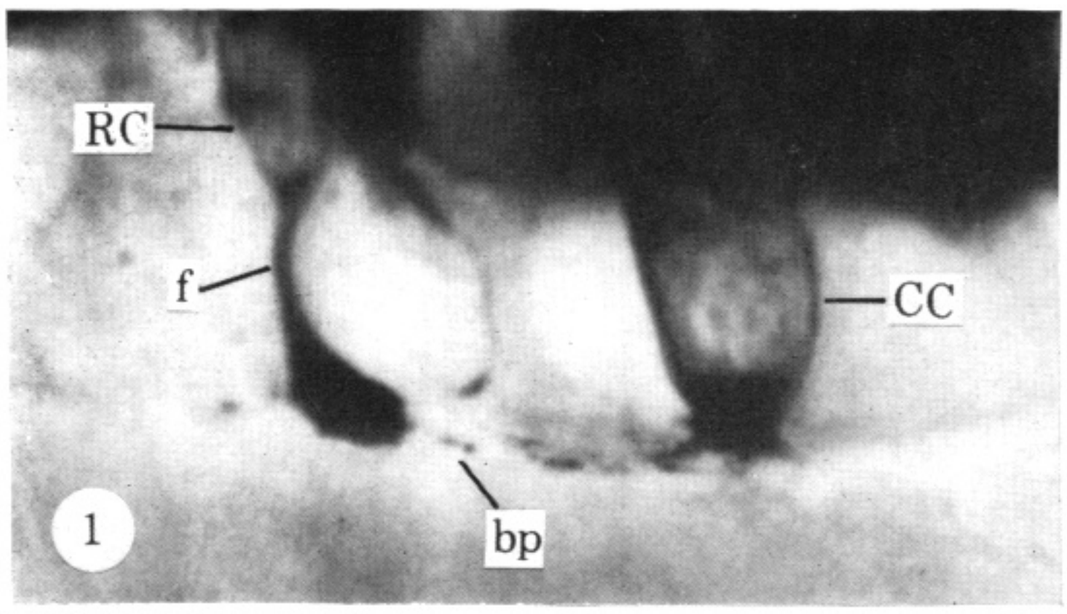
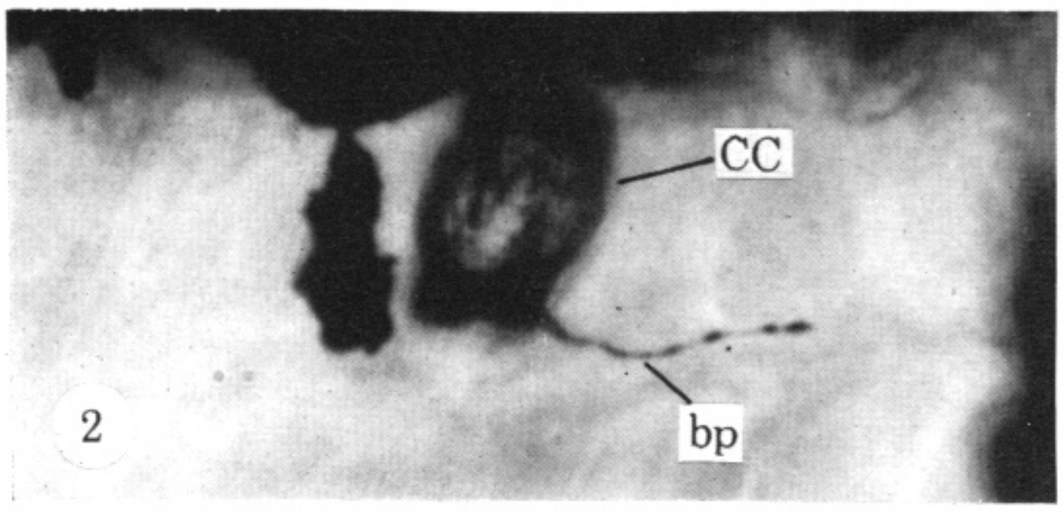
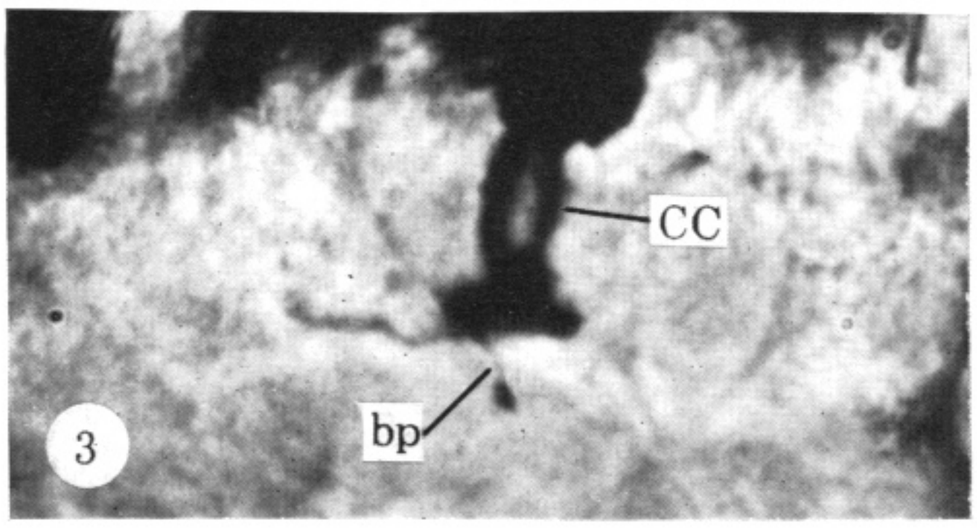
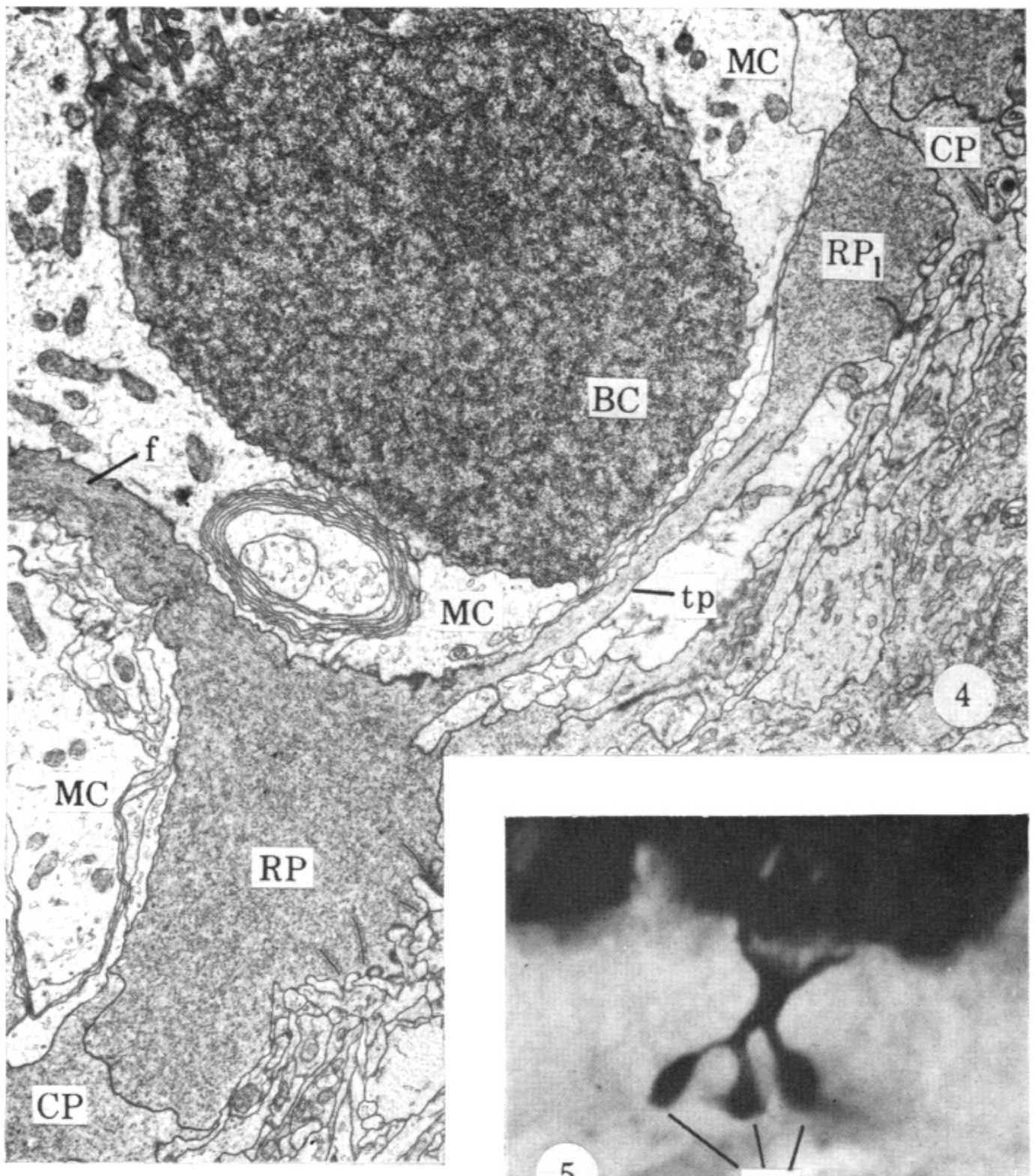
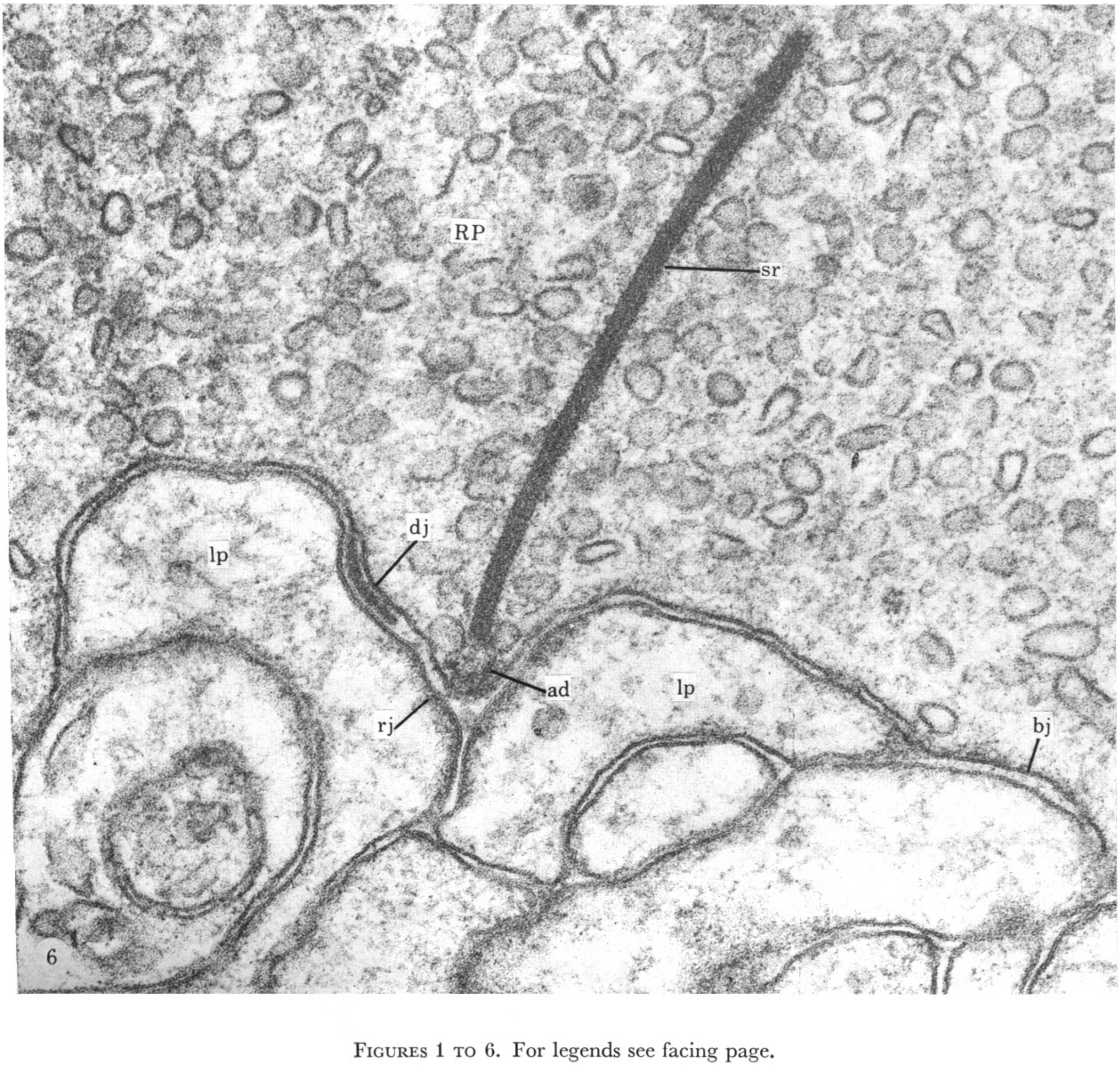


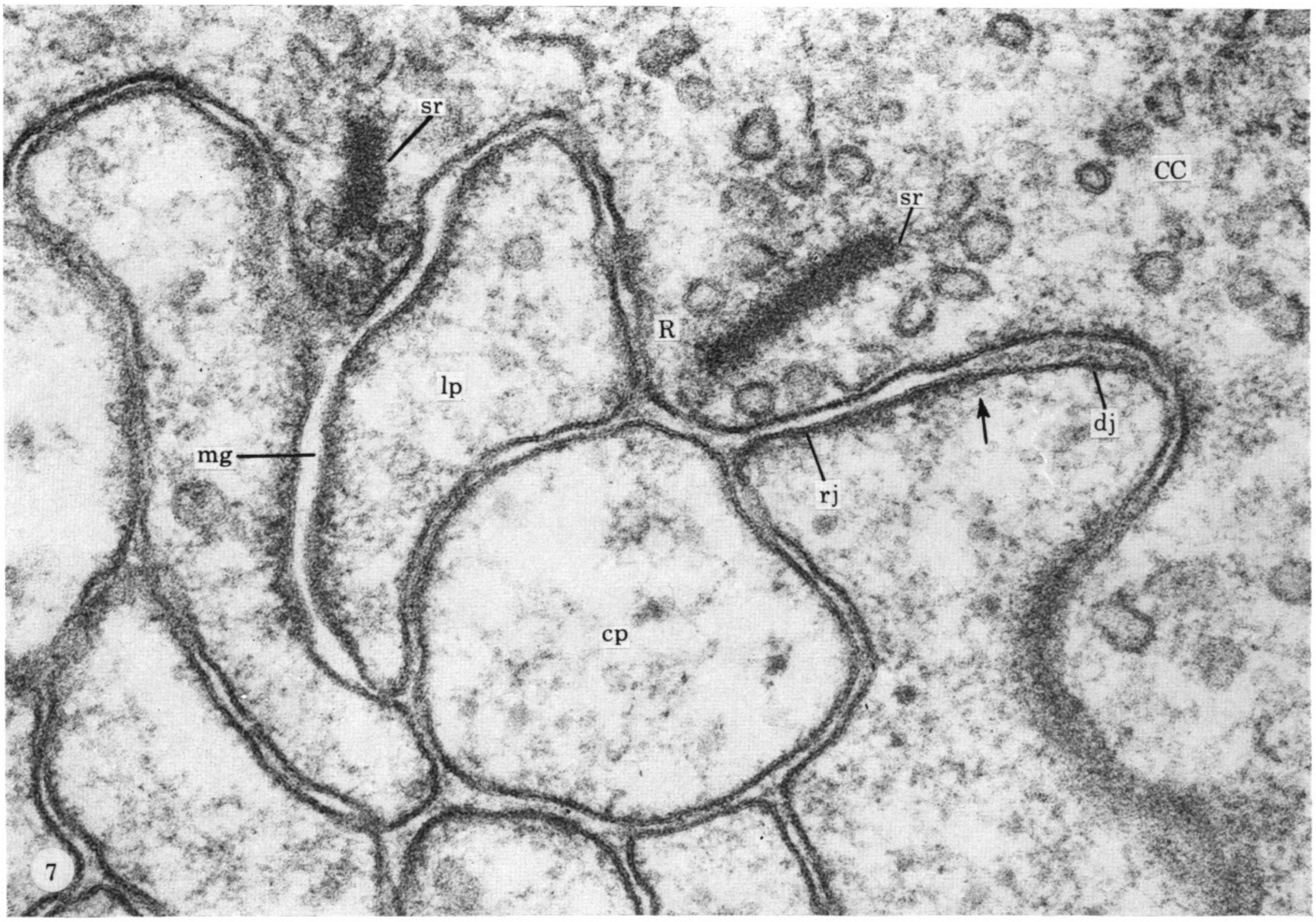


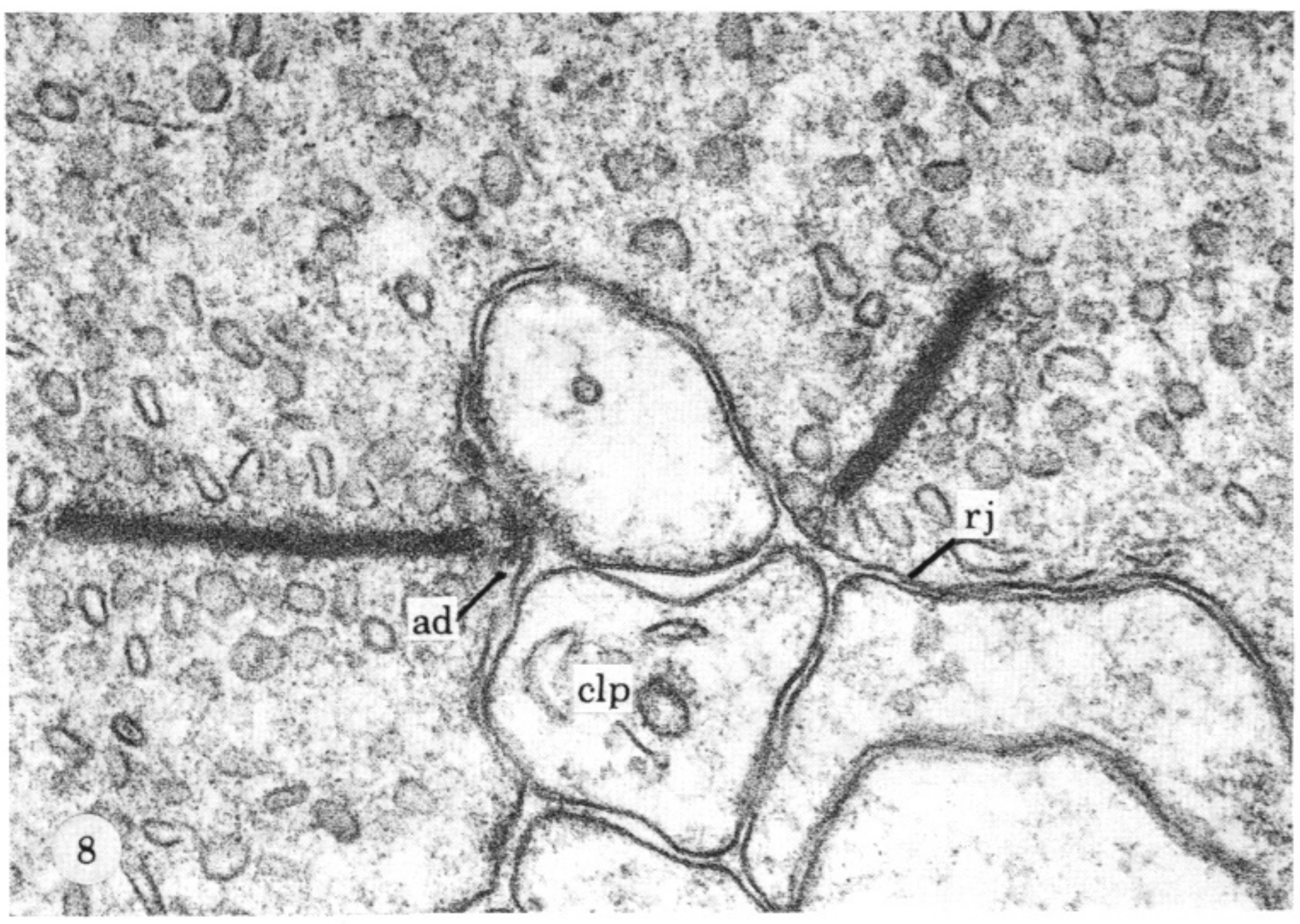


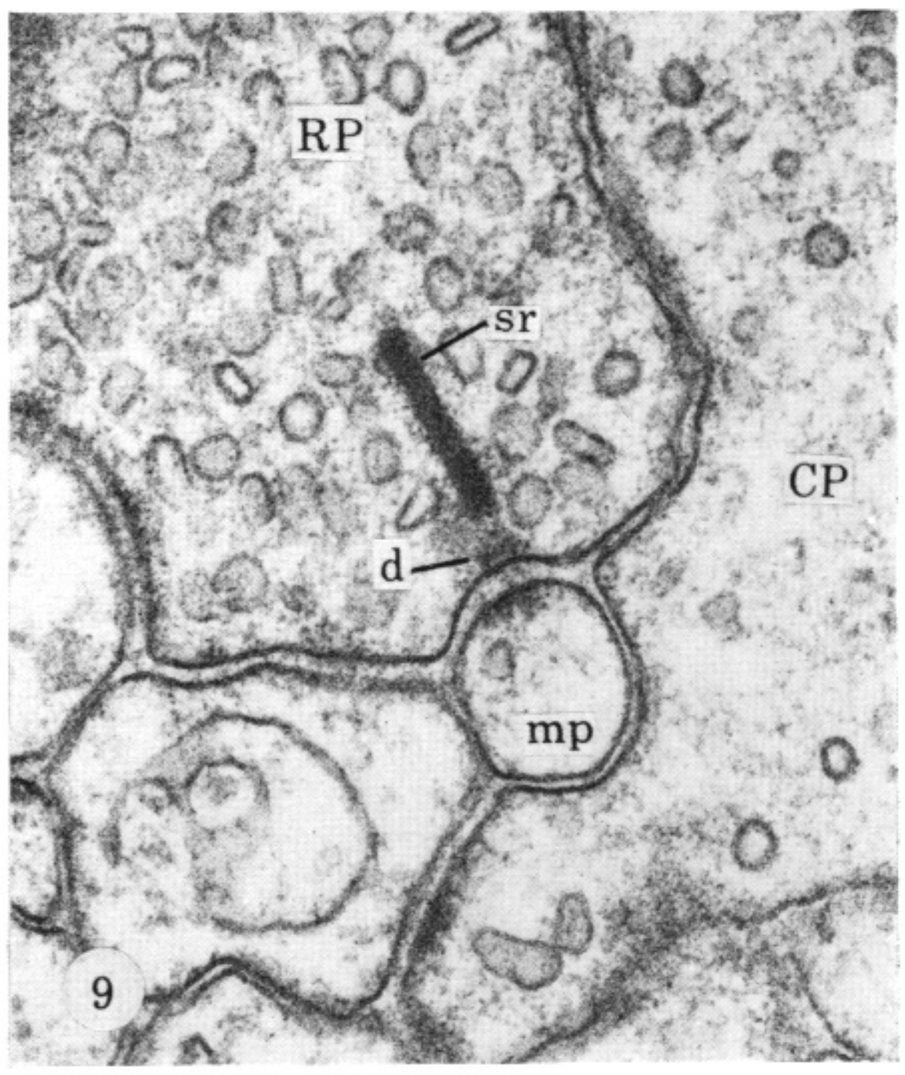


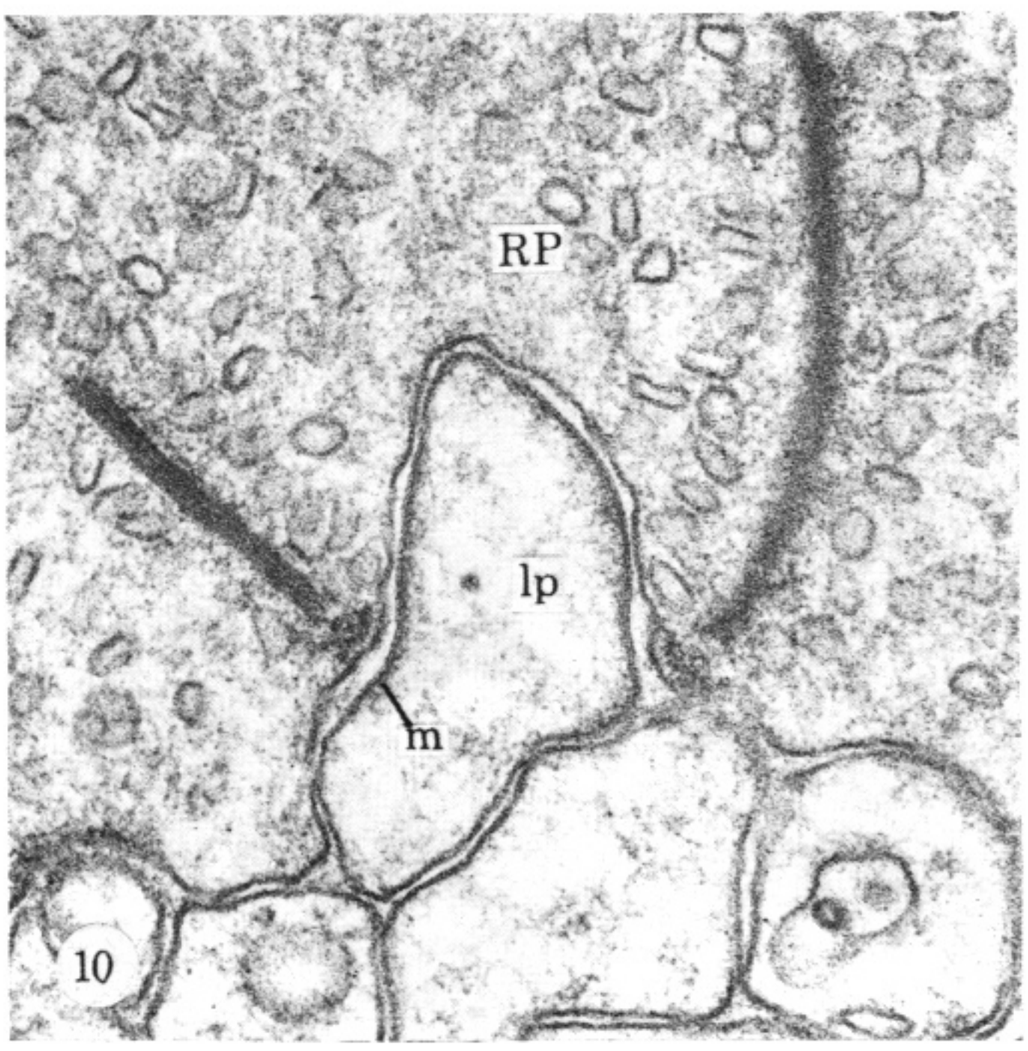


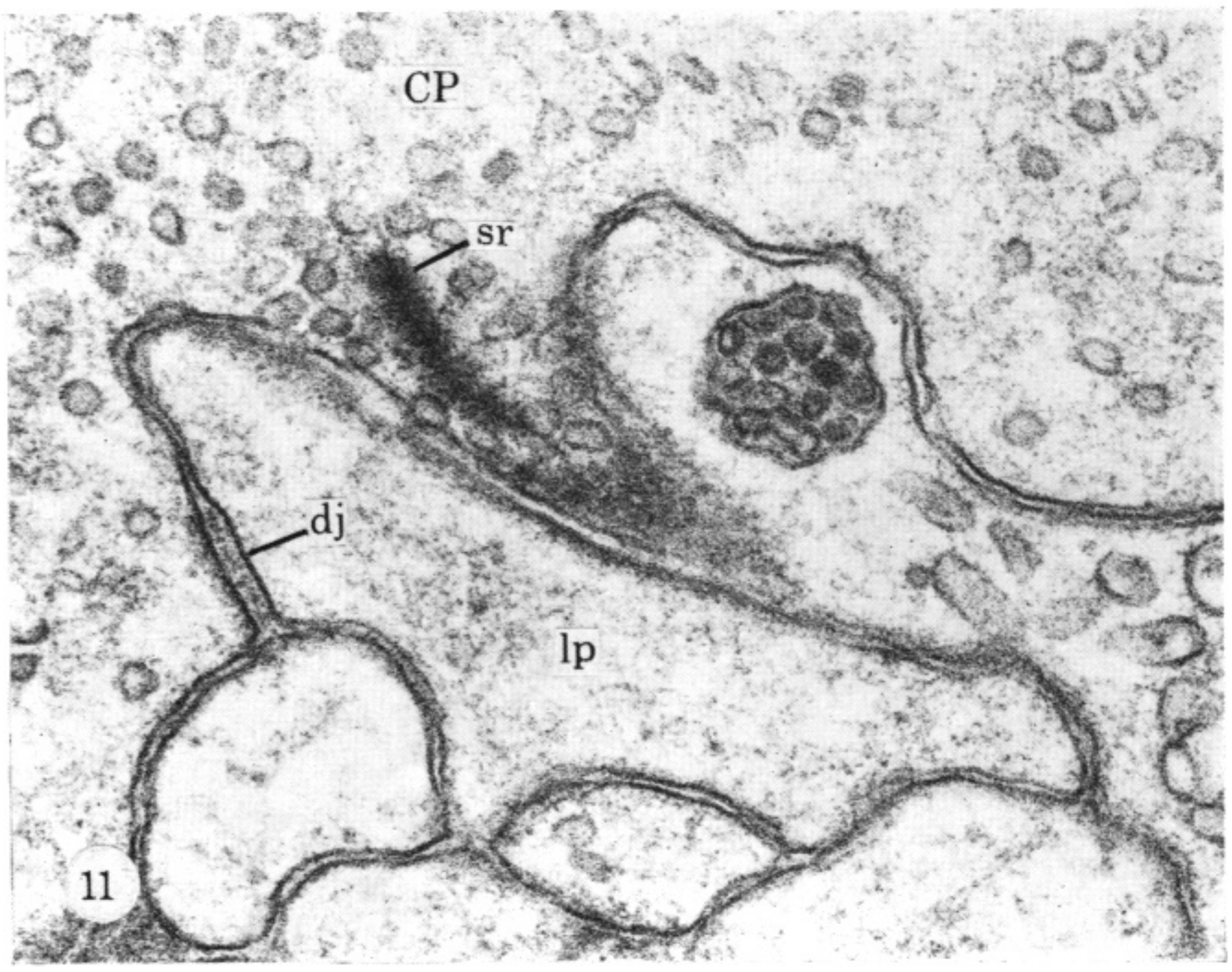
FIGURES 1 TO 6. For legends see facing page.











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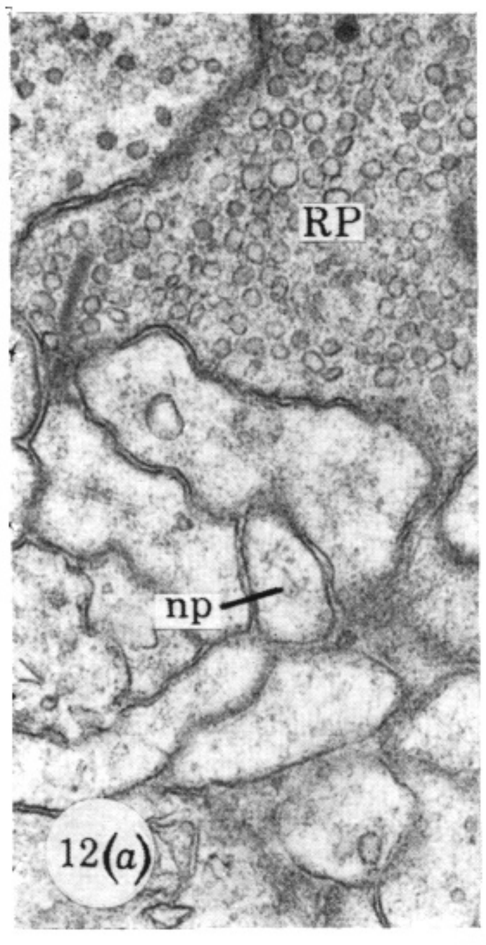

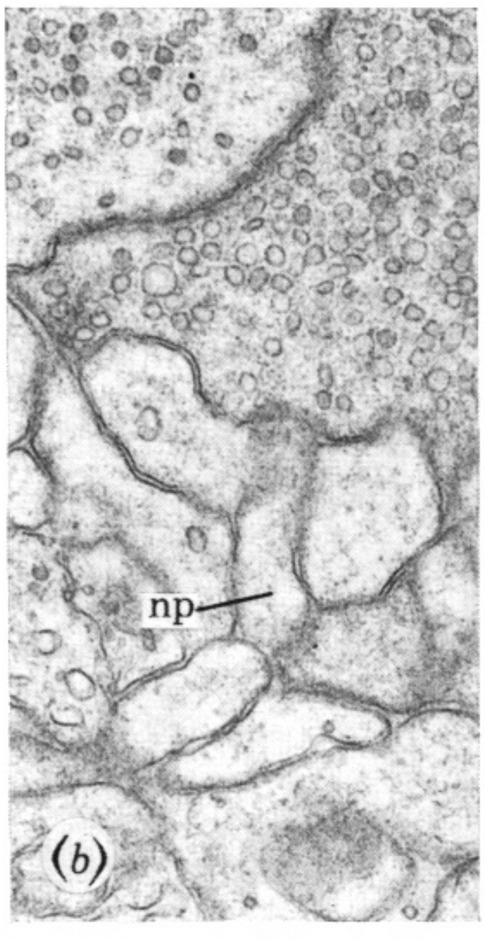

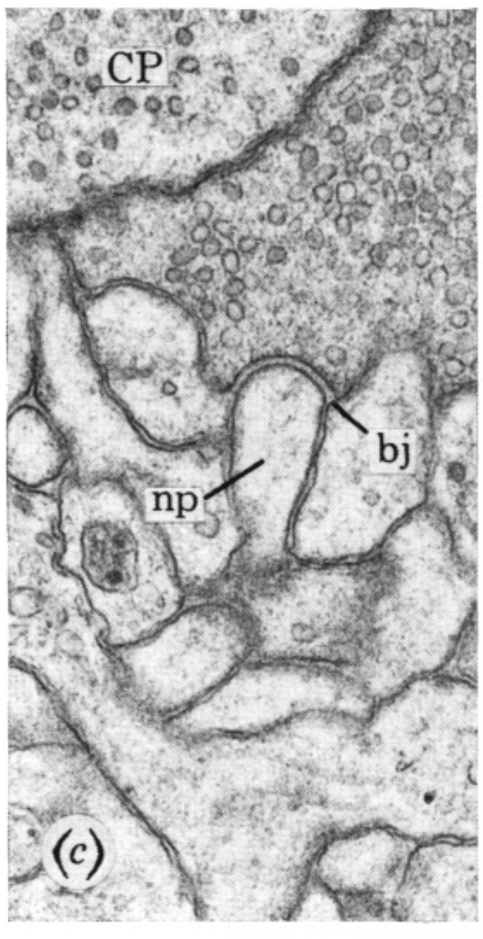

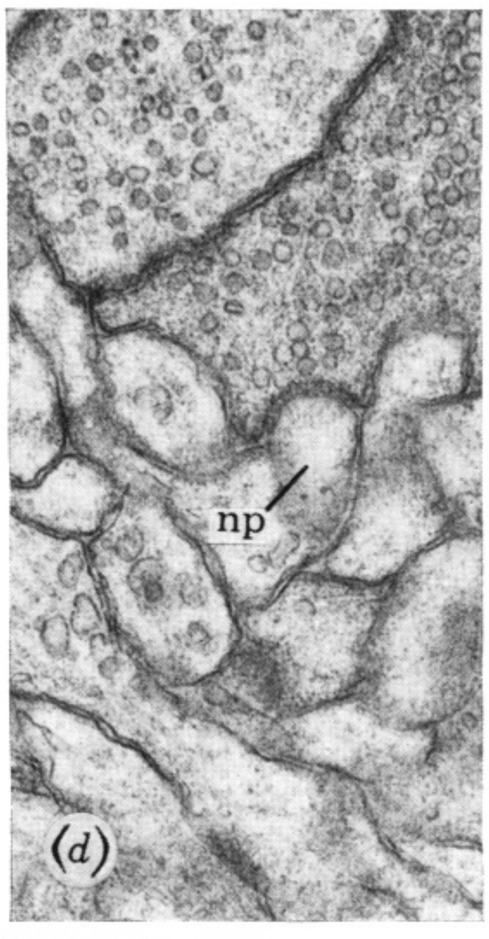

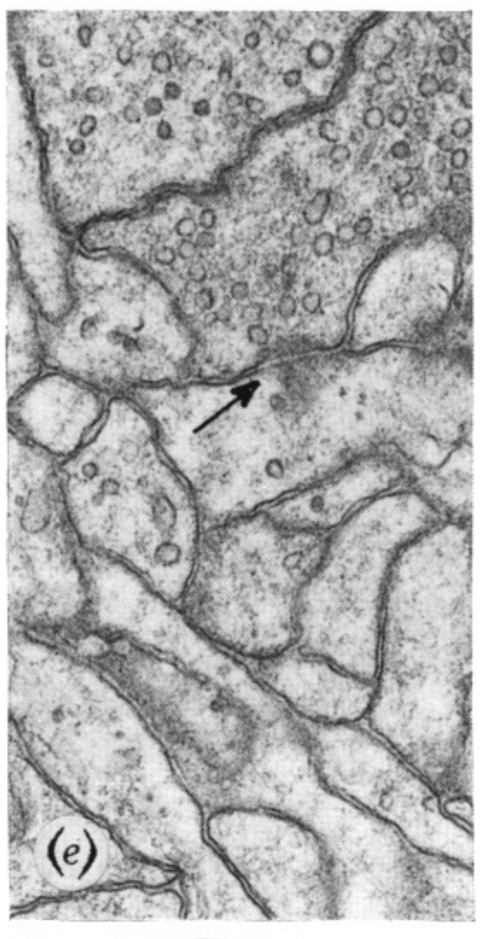

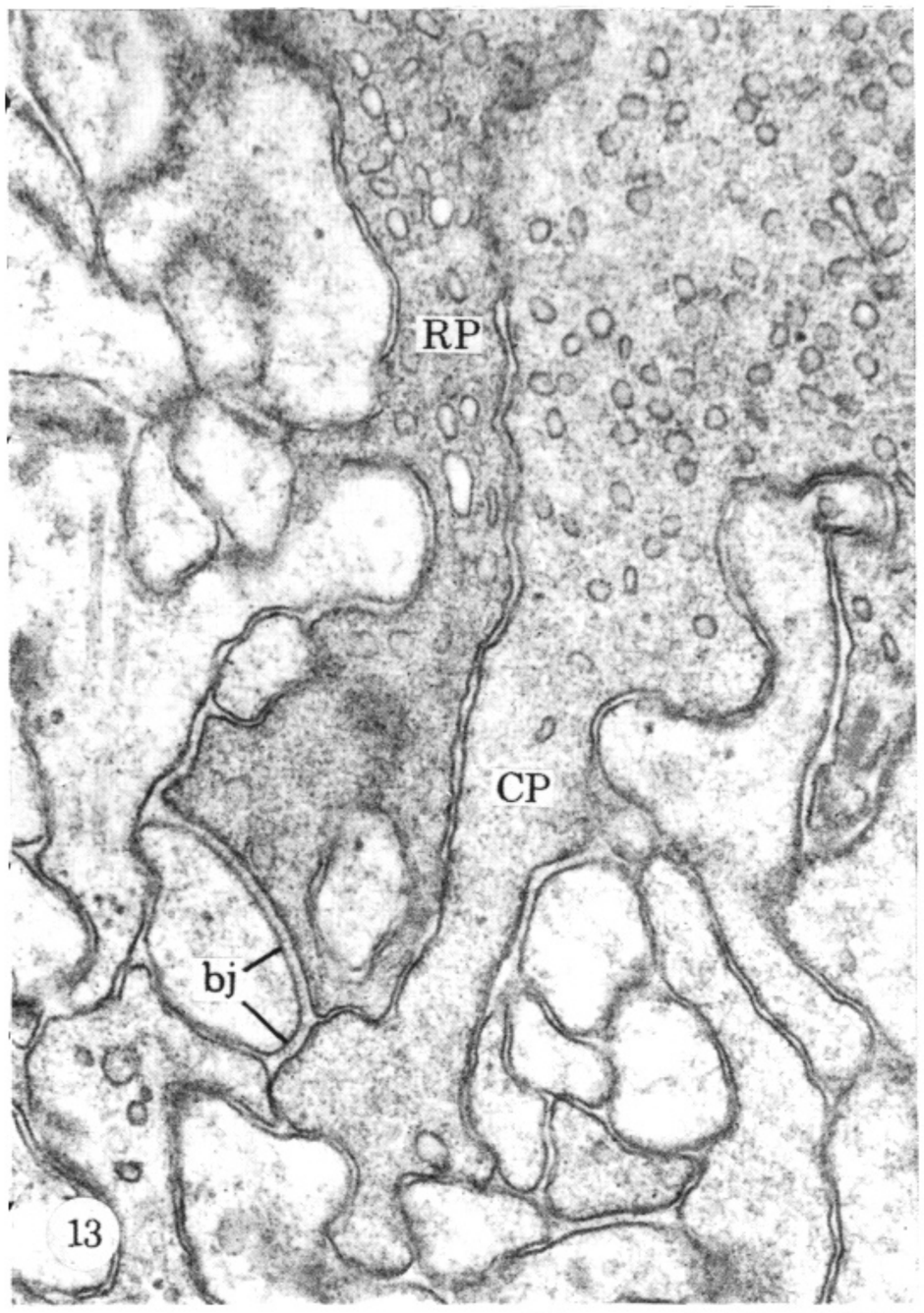

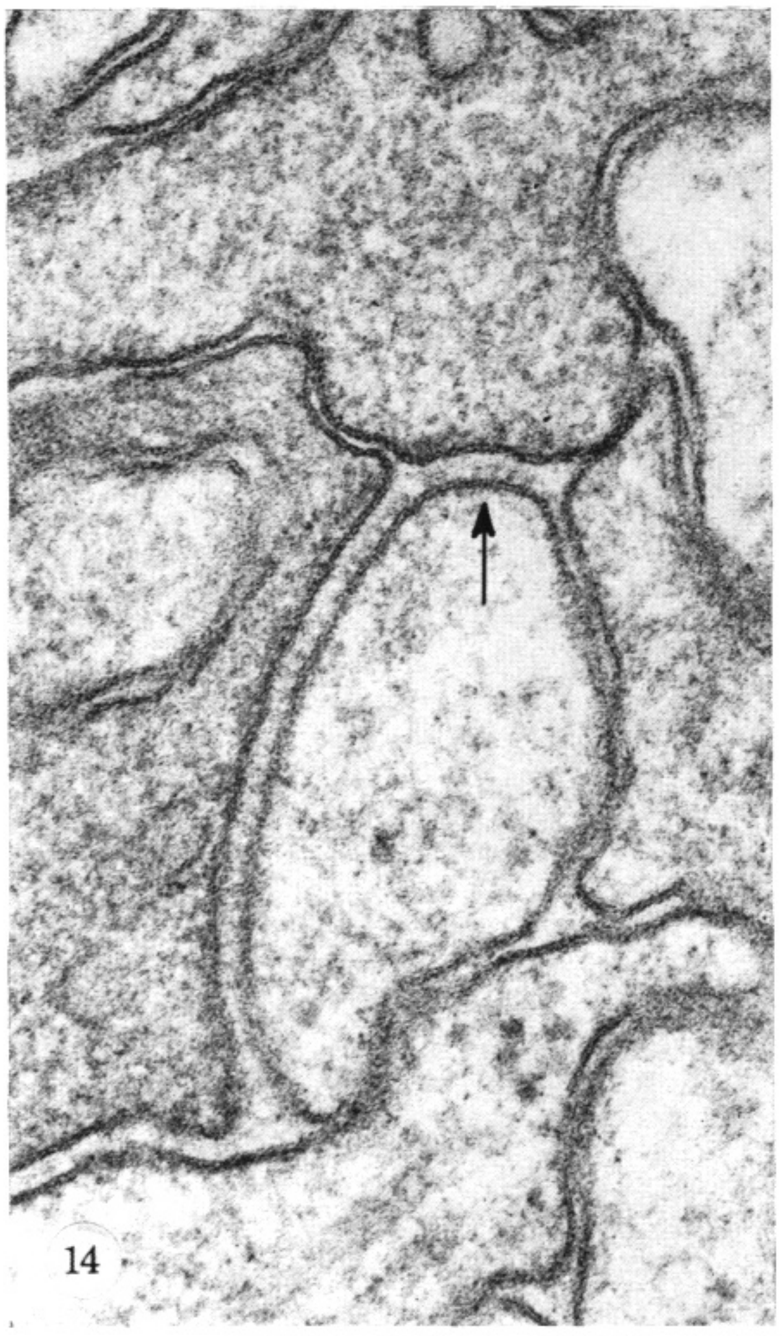

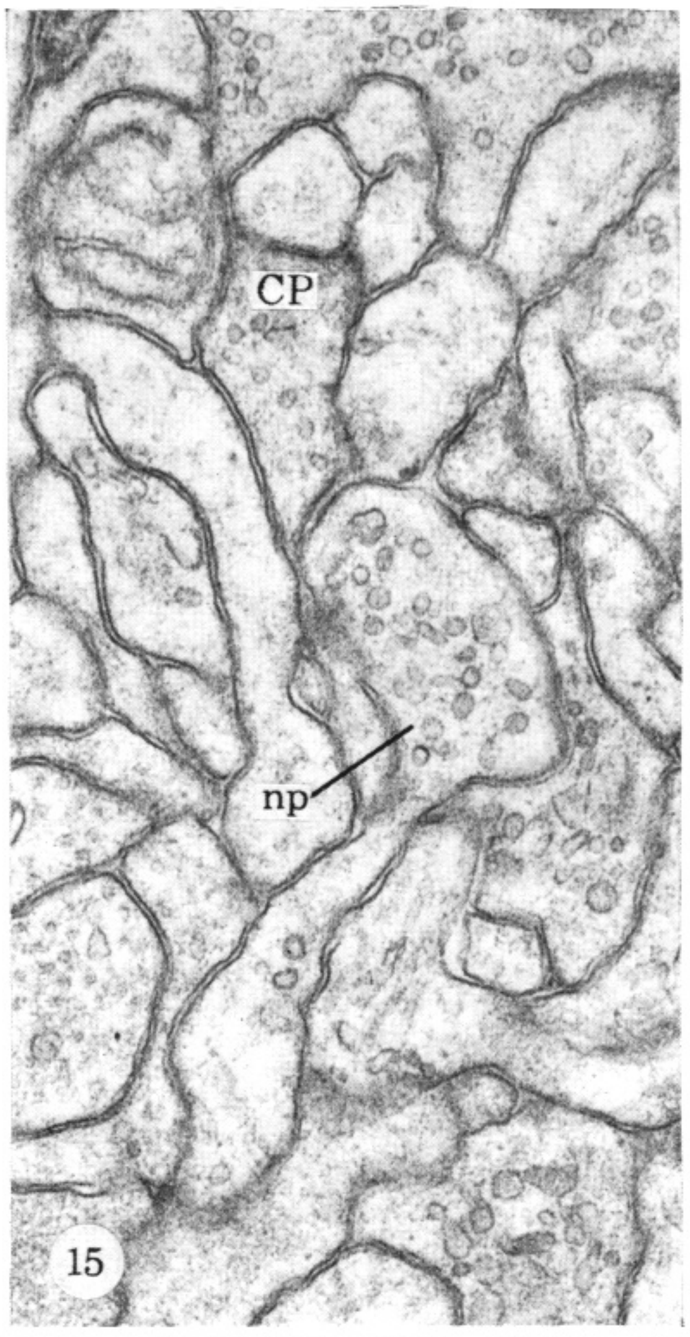

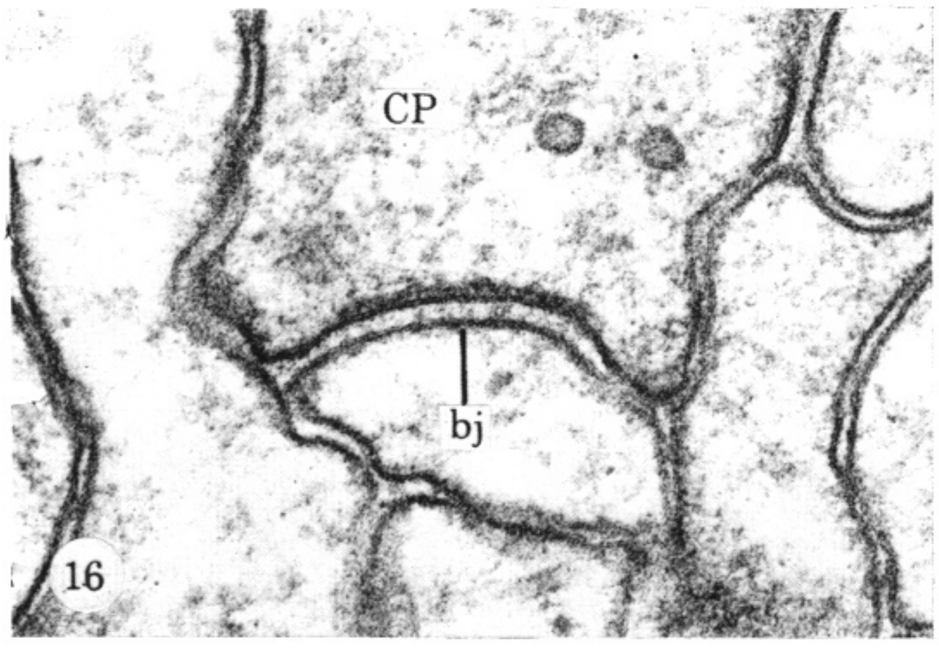

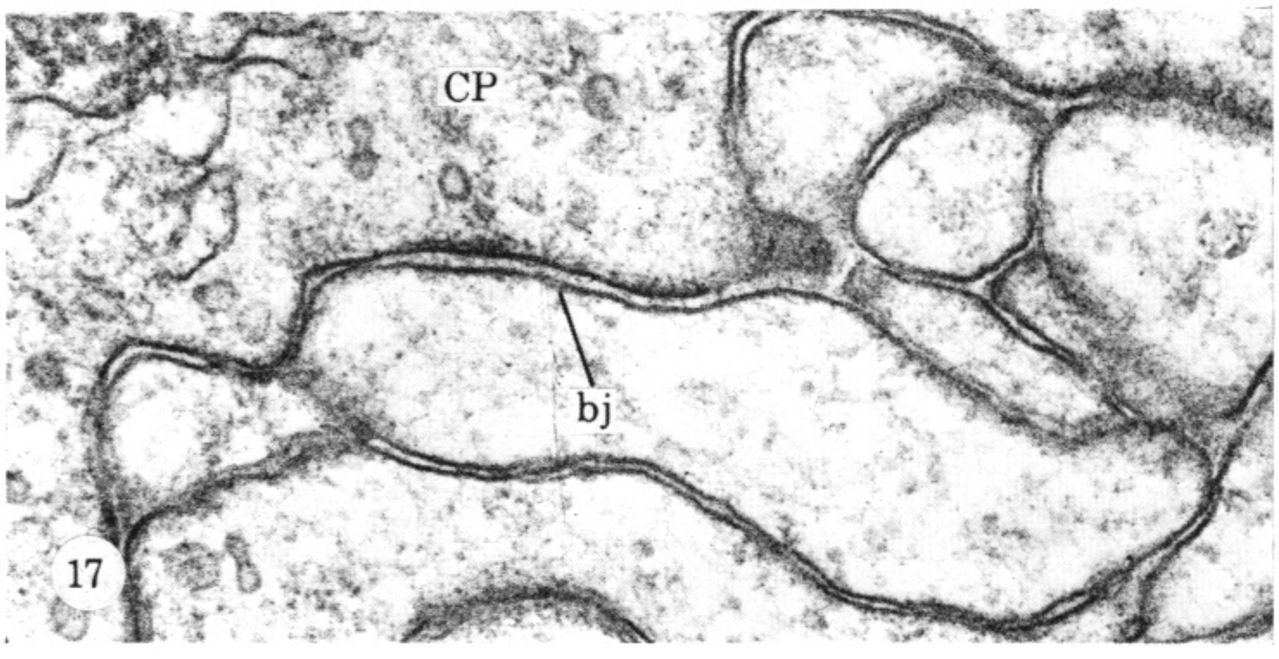

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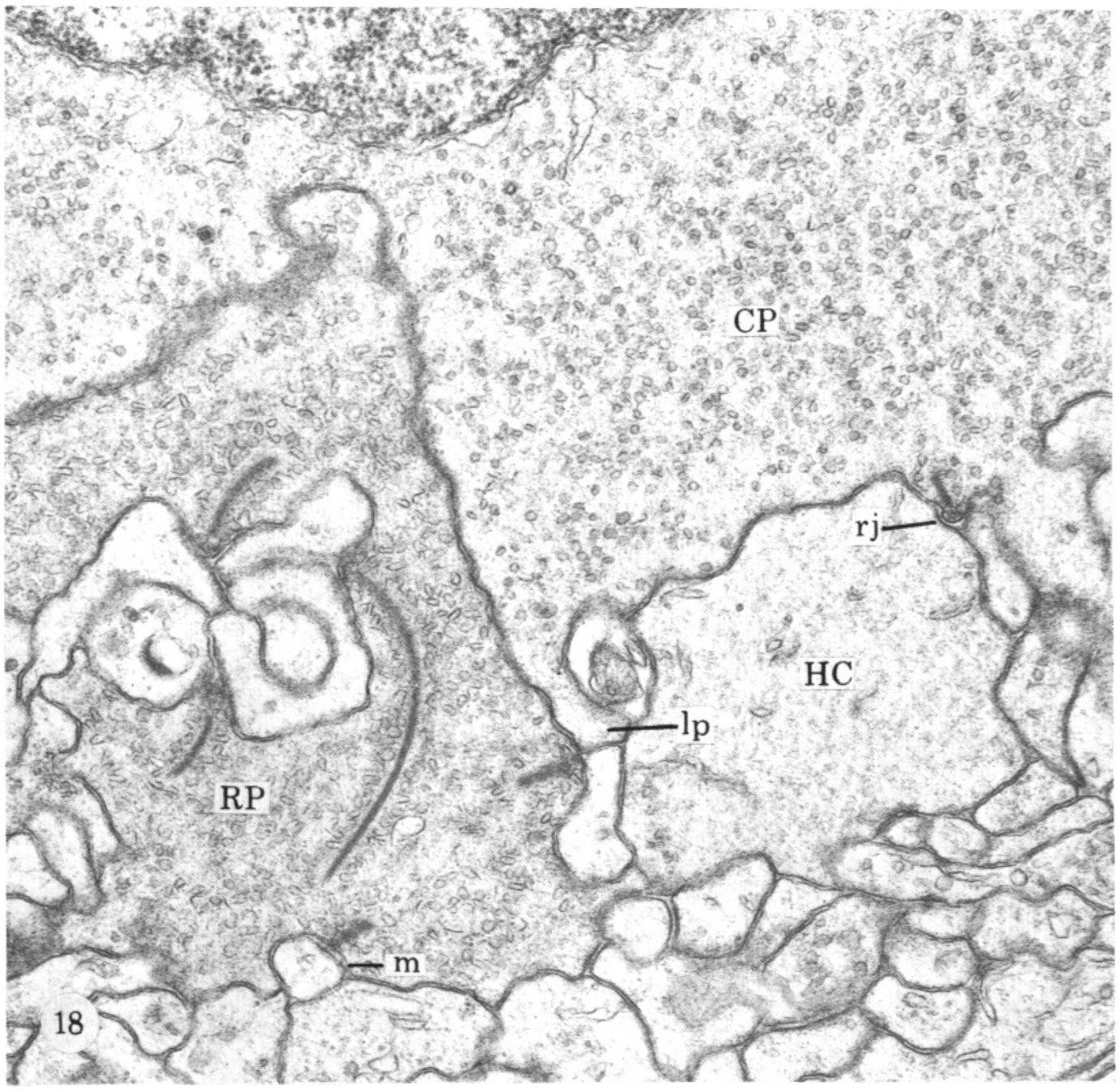

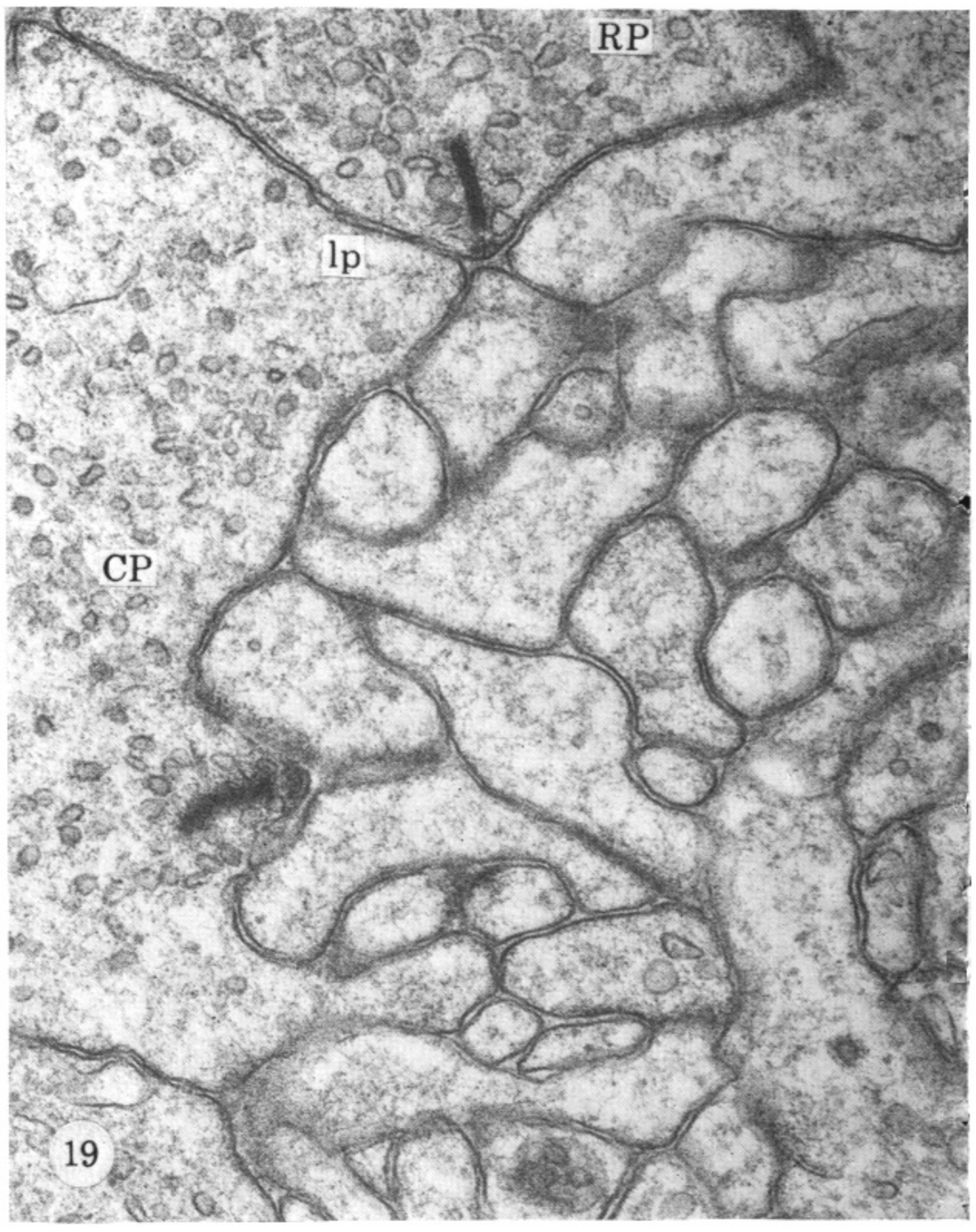

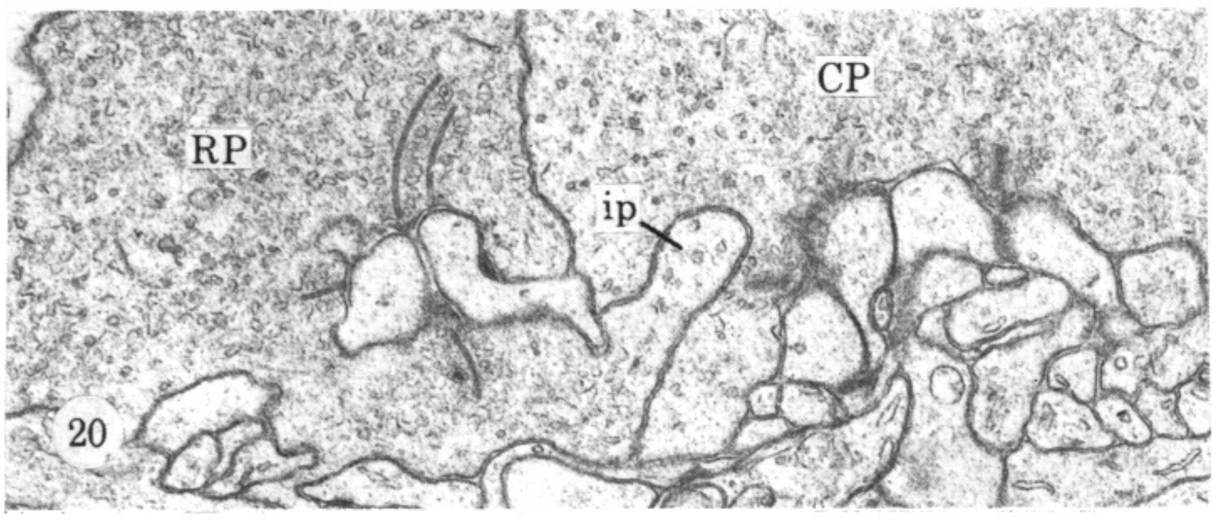

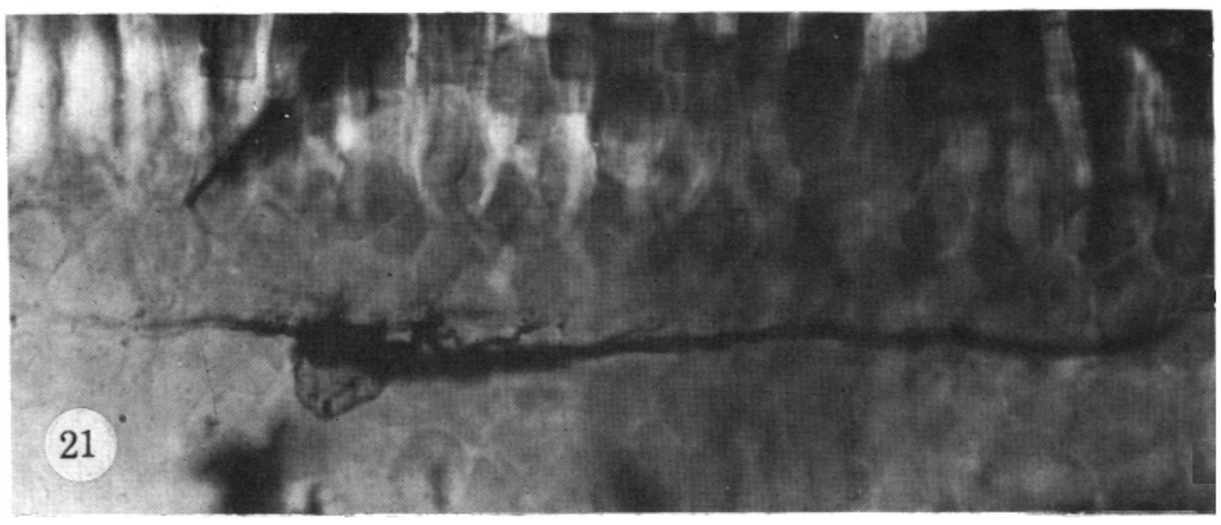

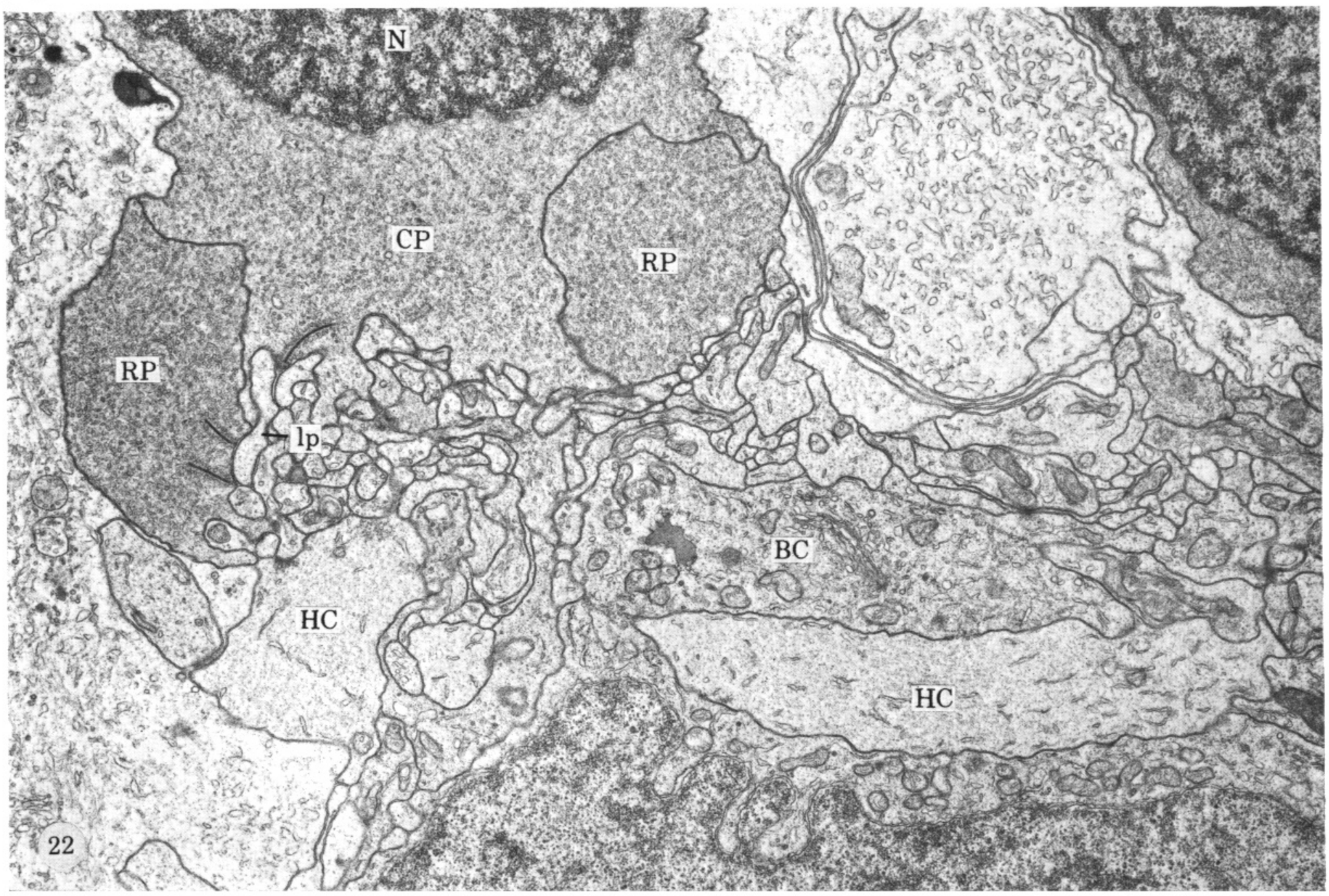

FIGURES 18 TO 22. For legends see facing page.

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HC
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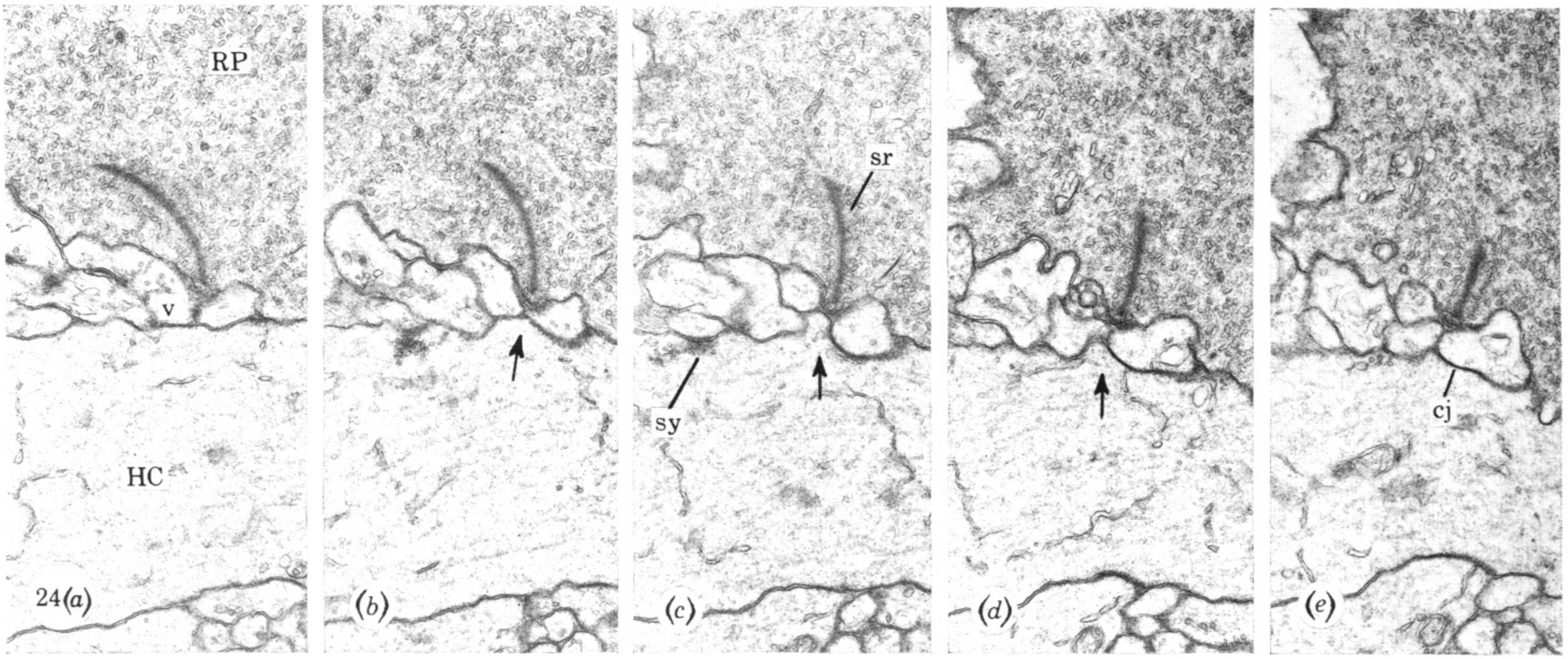
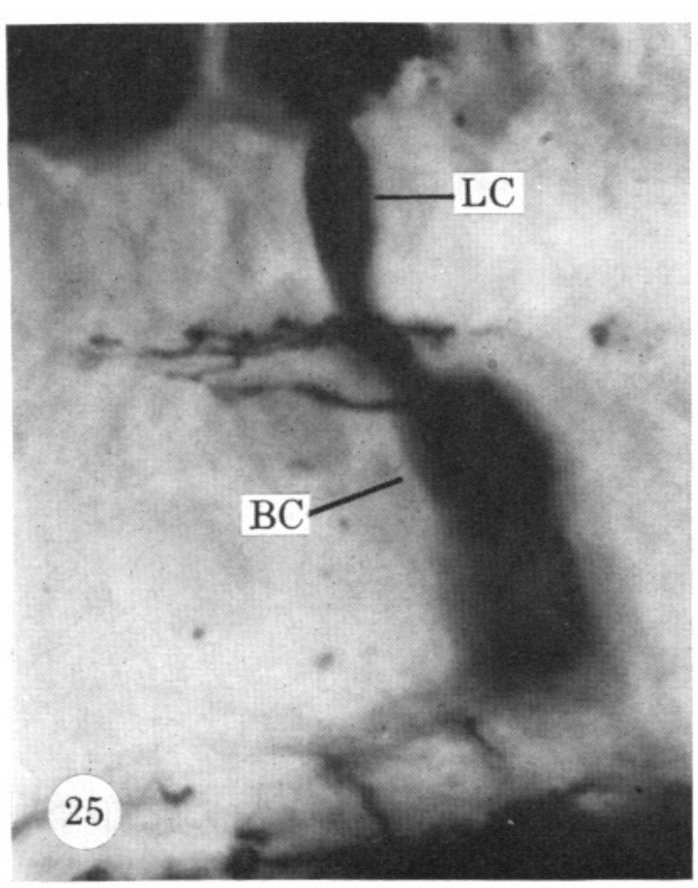
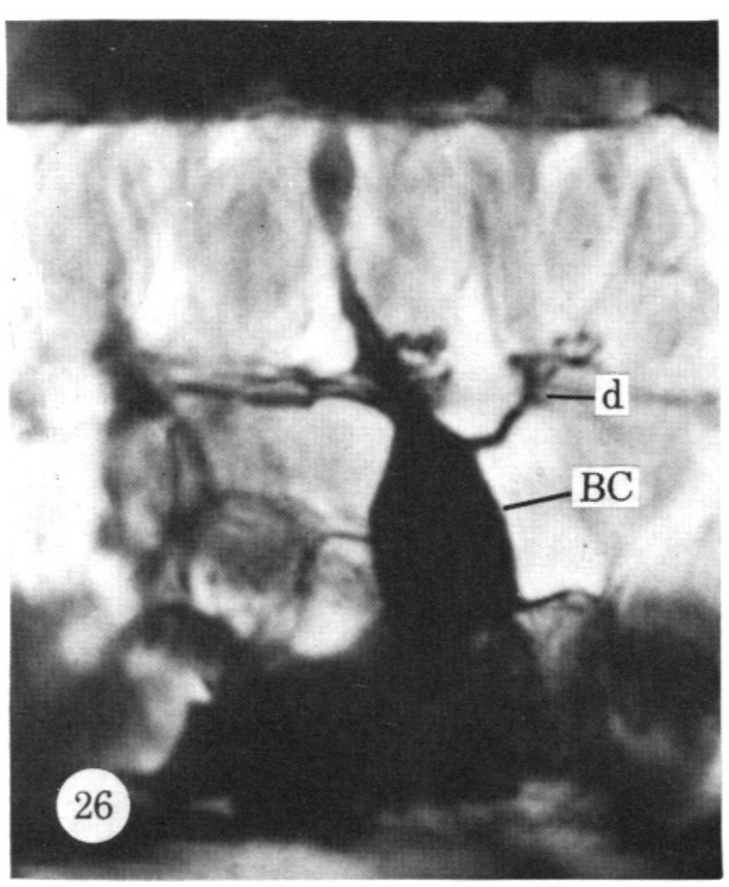
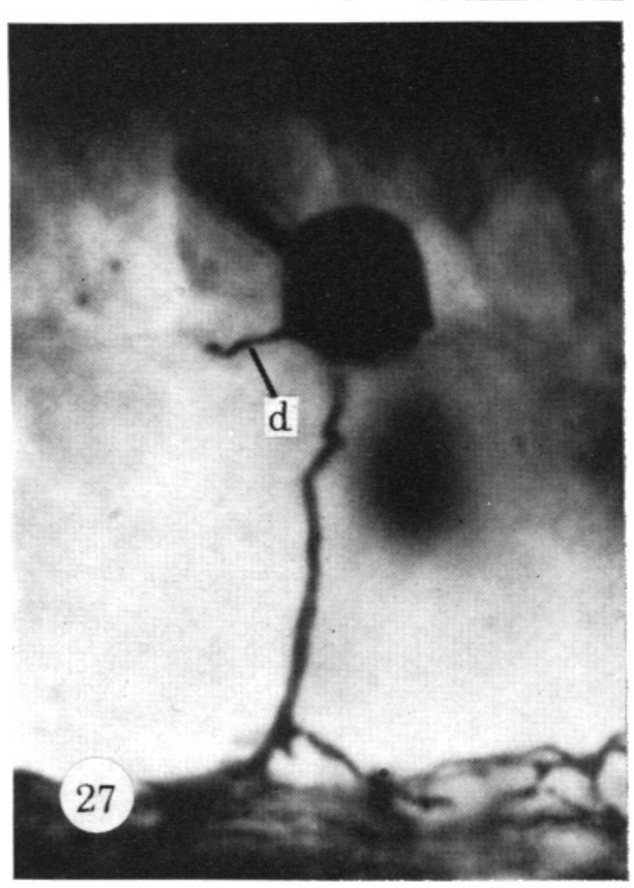
(c)

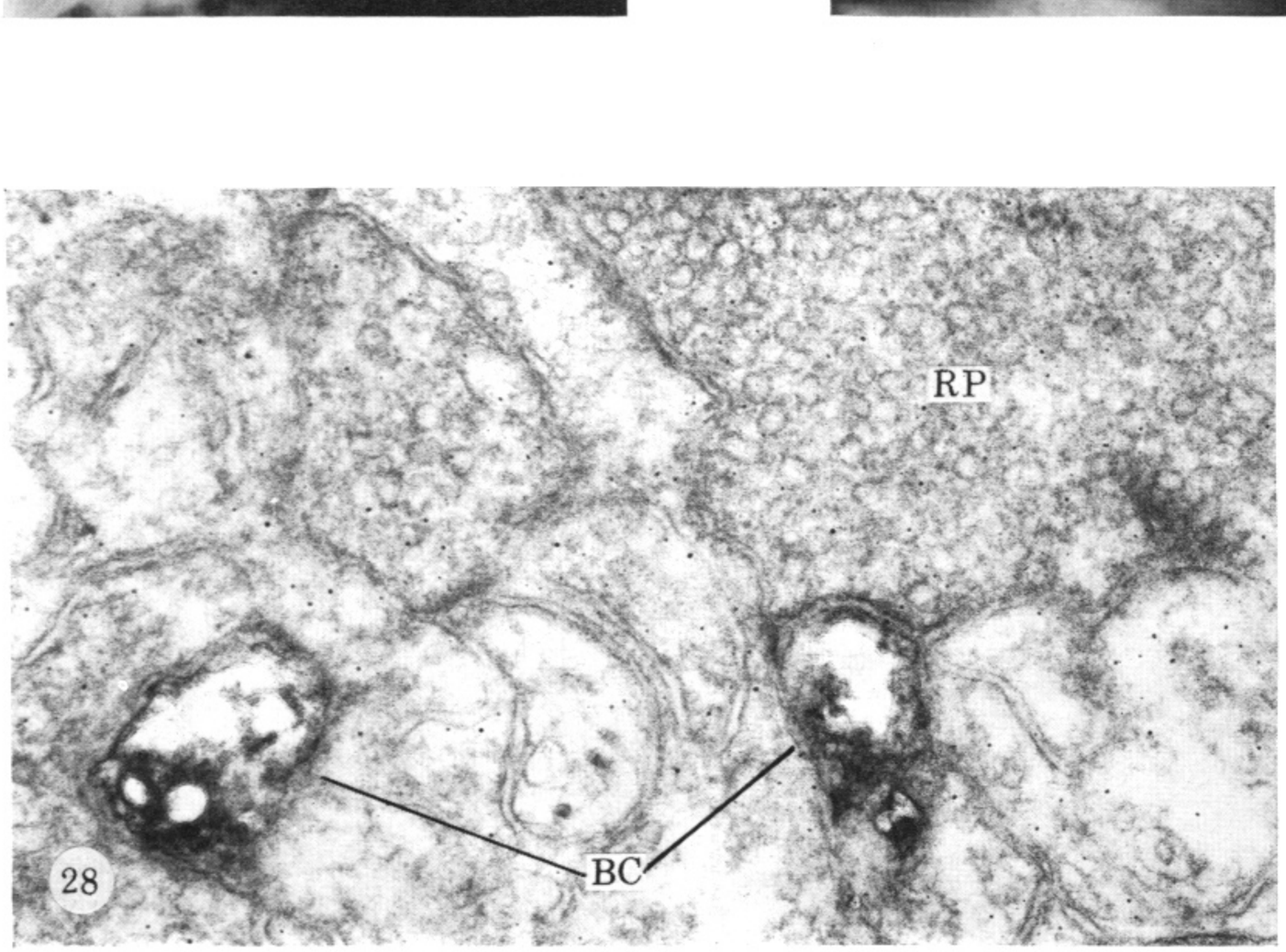
FIGURE 23. For legends see facing page.

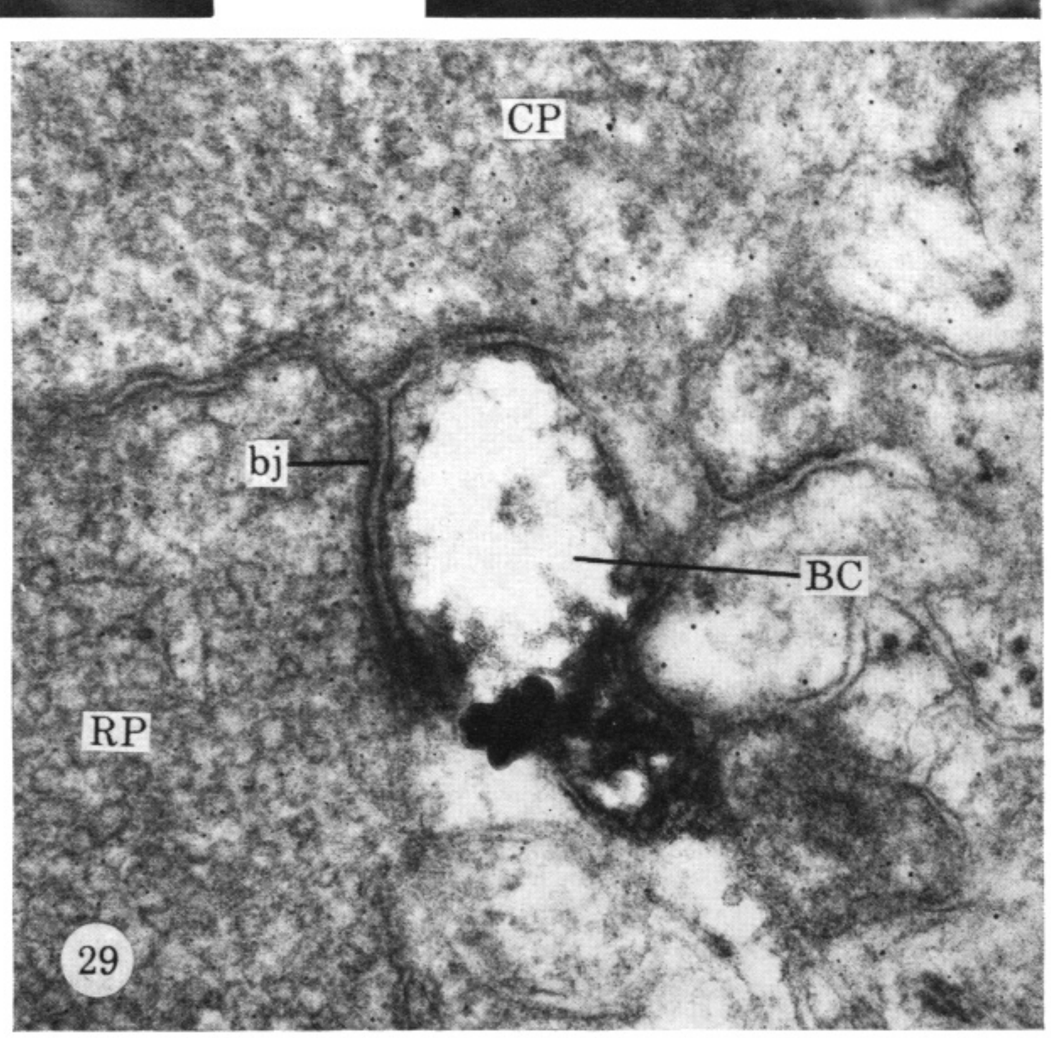












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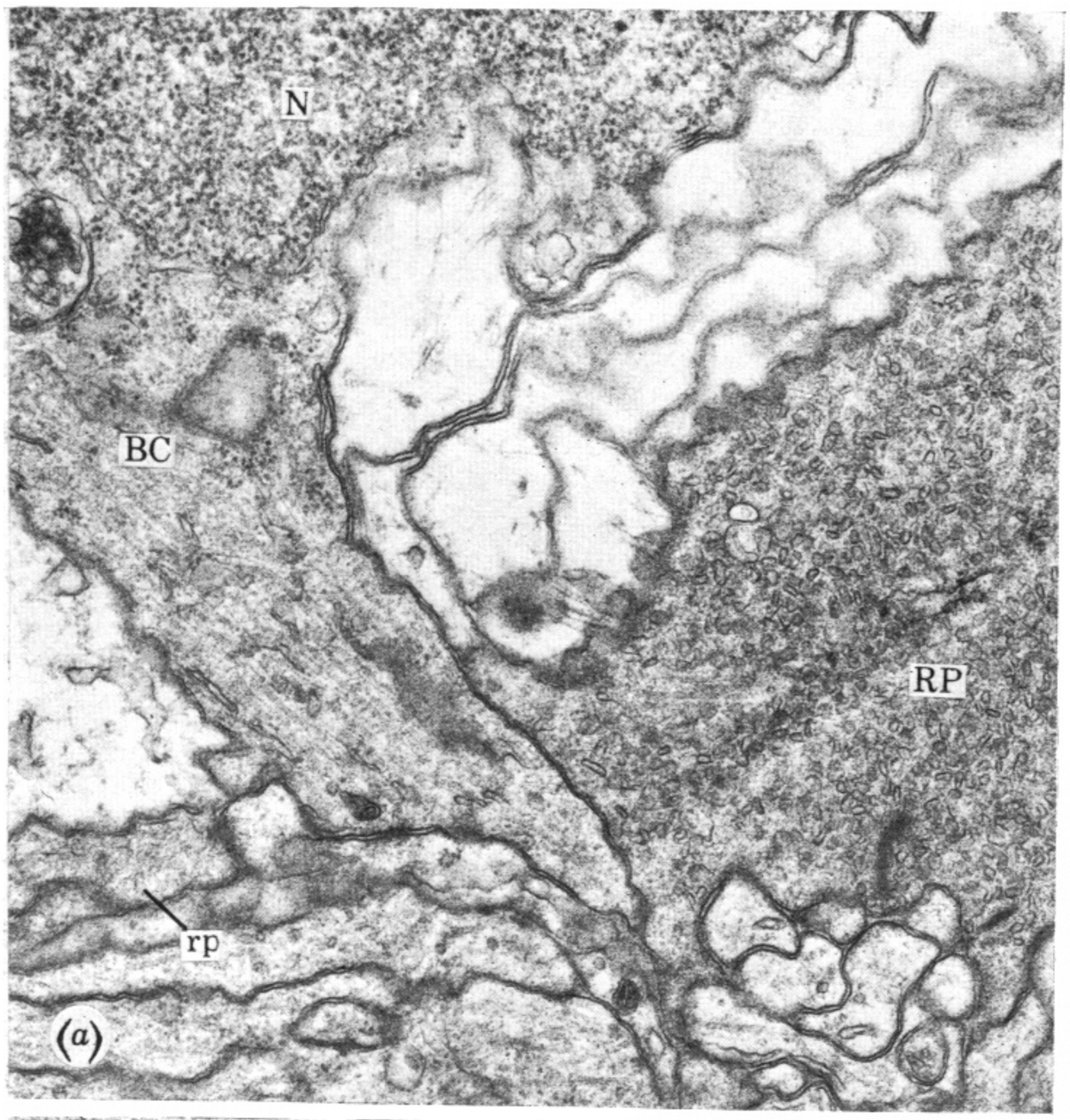

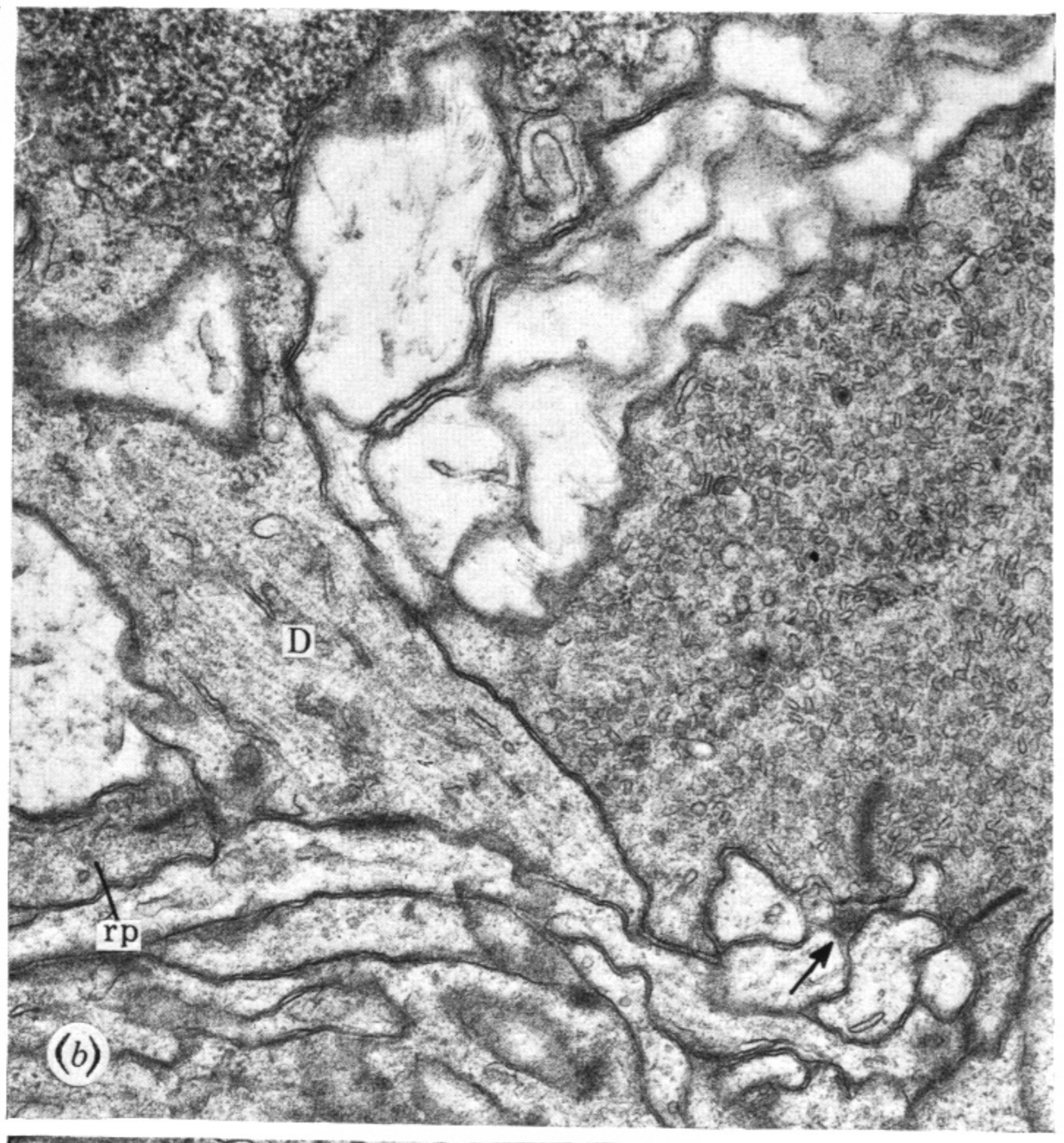

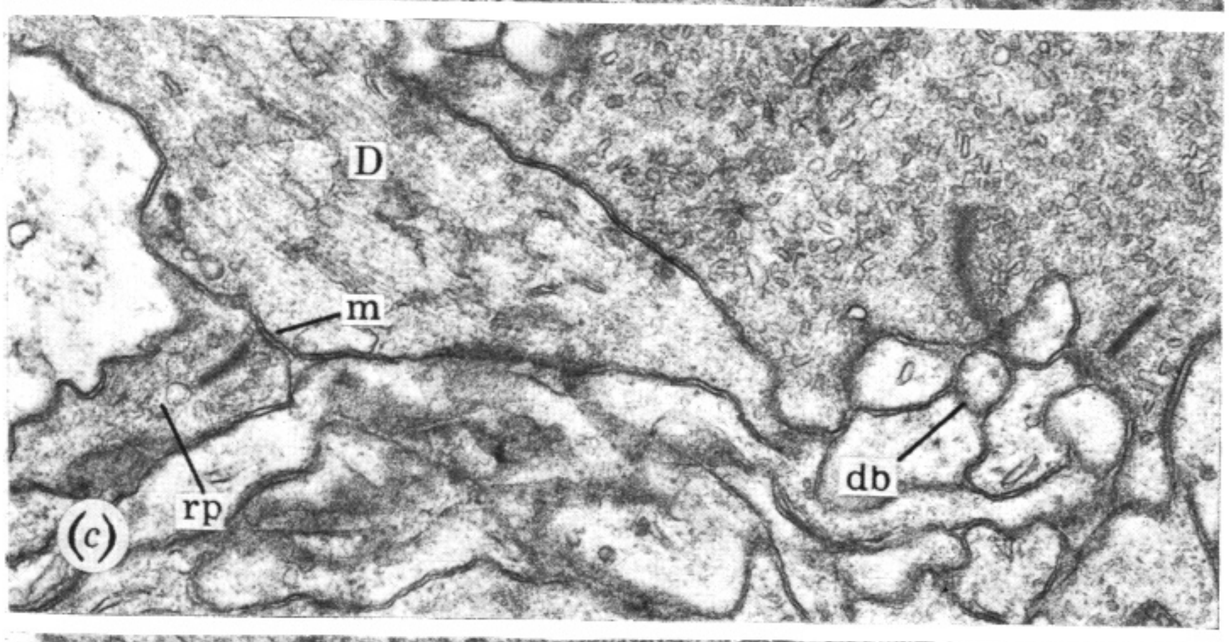

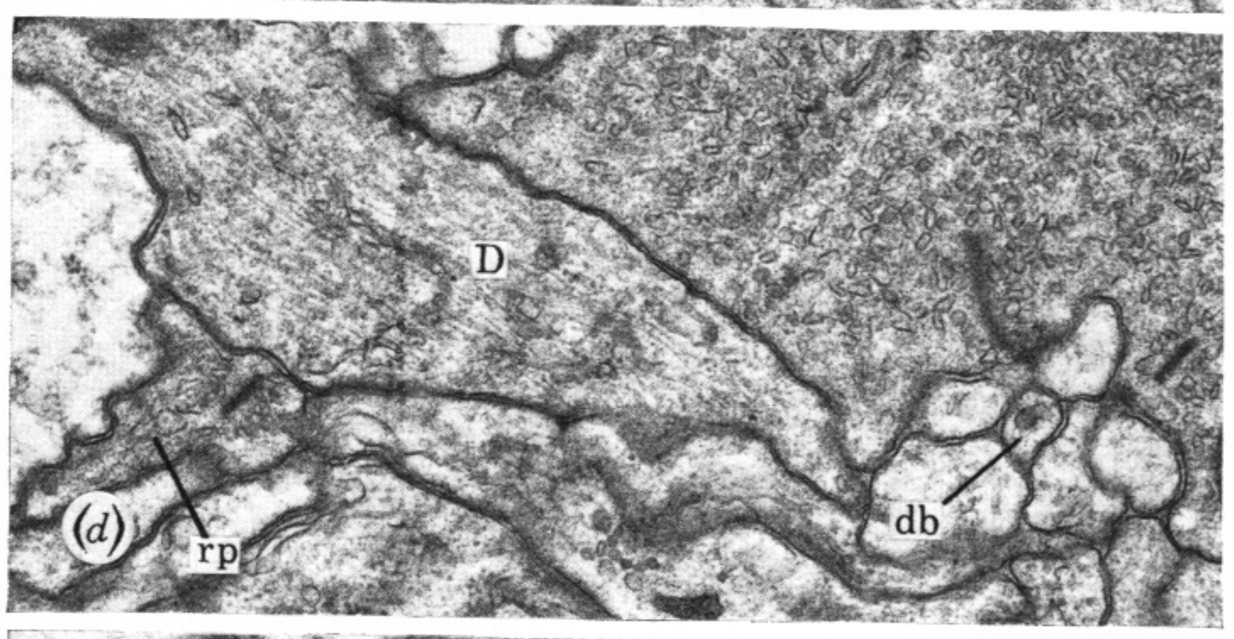

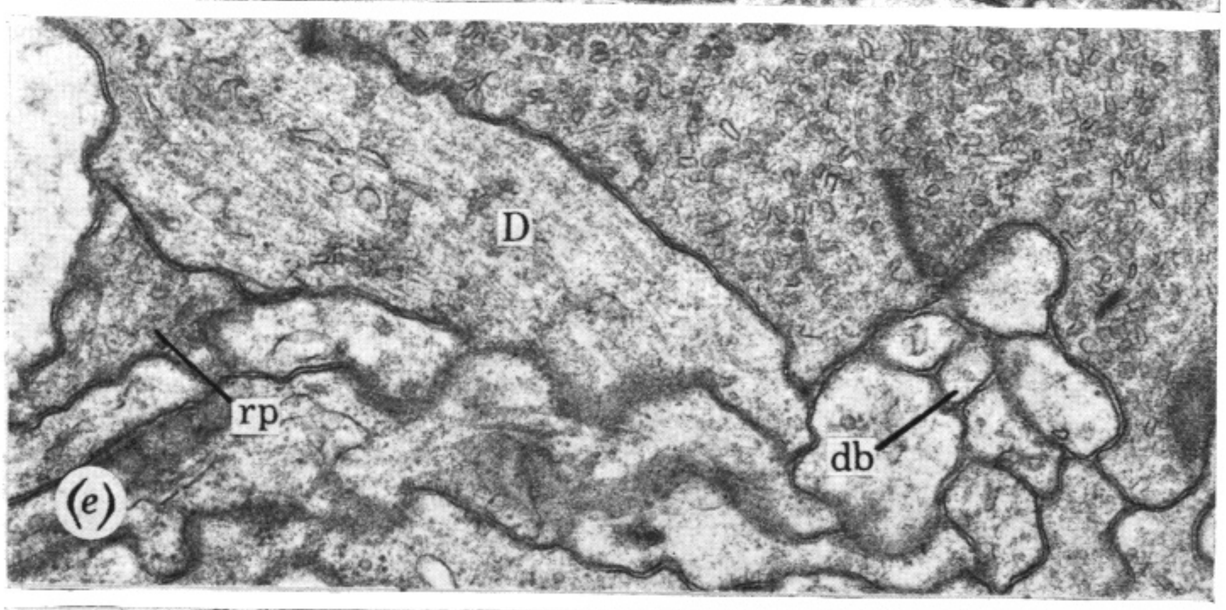

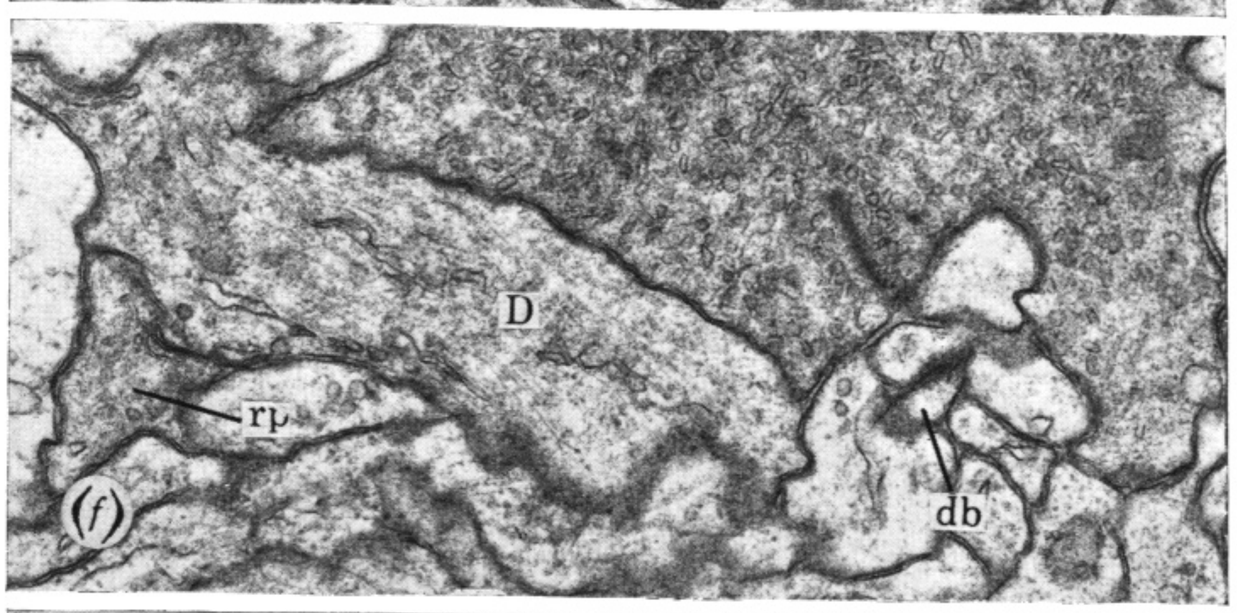

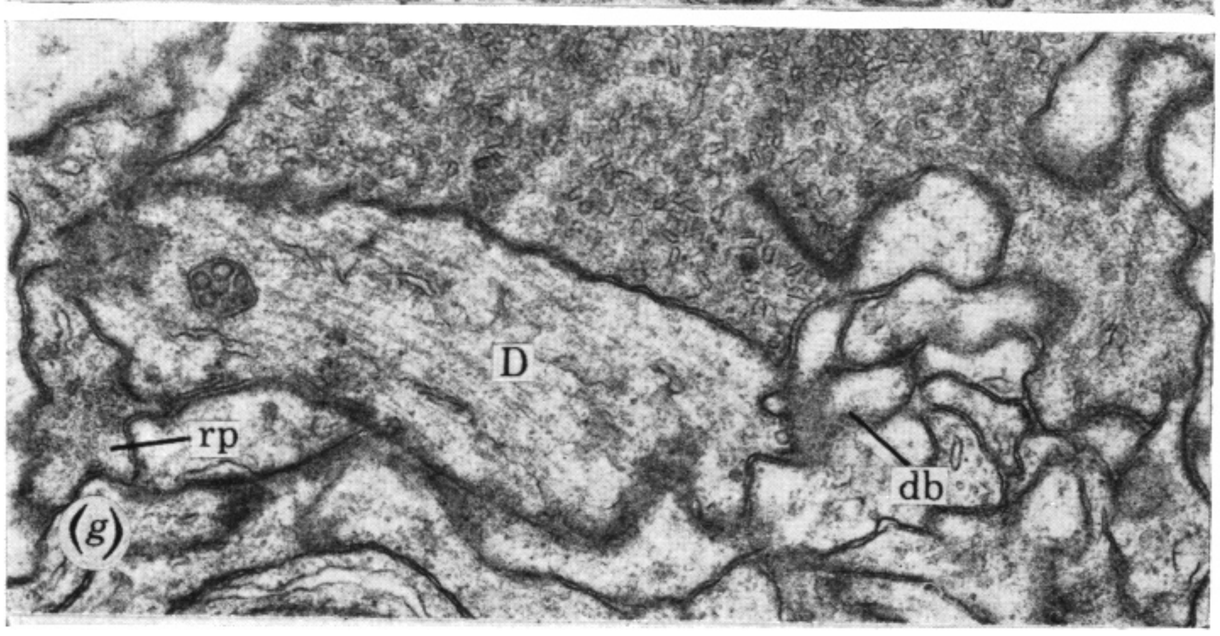

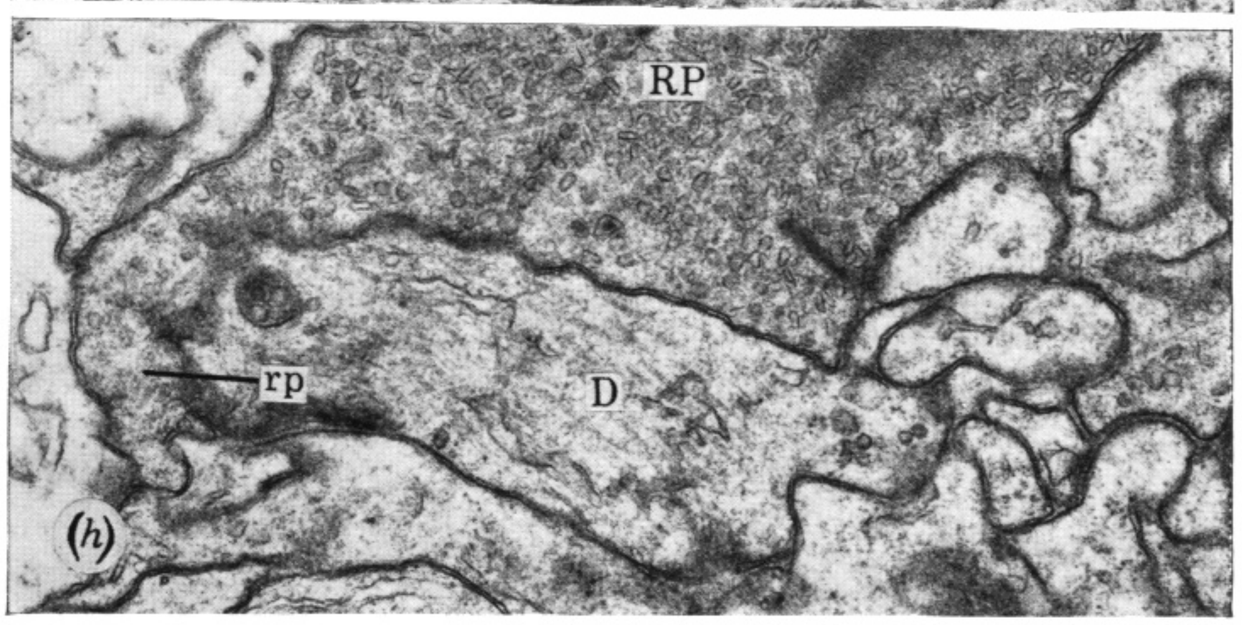

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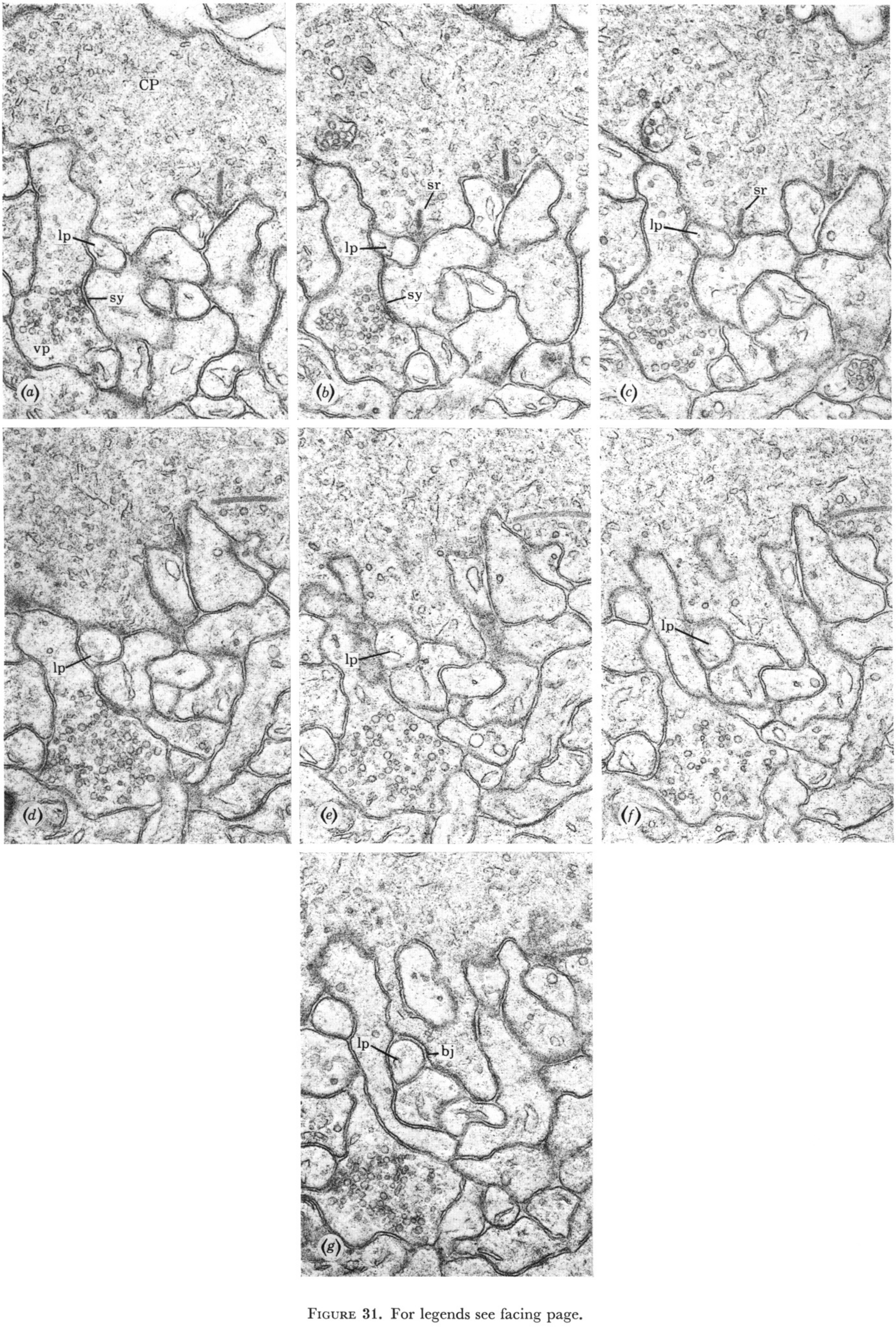

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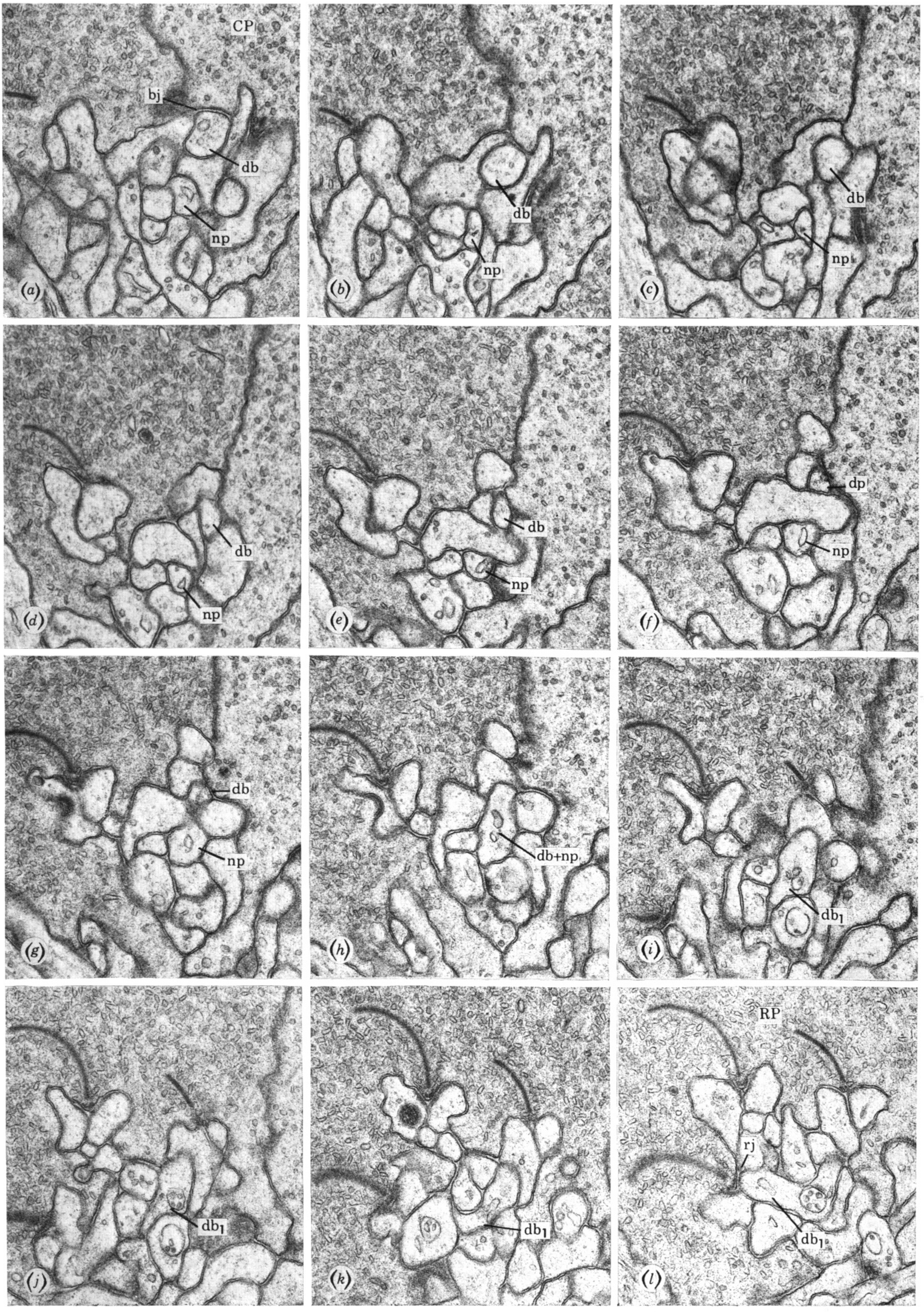

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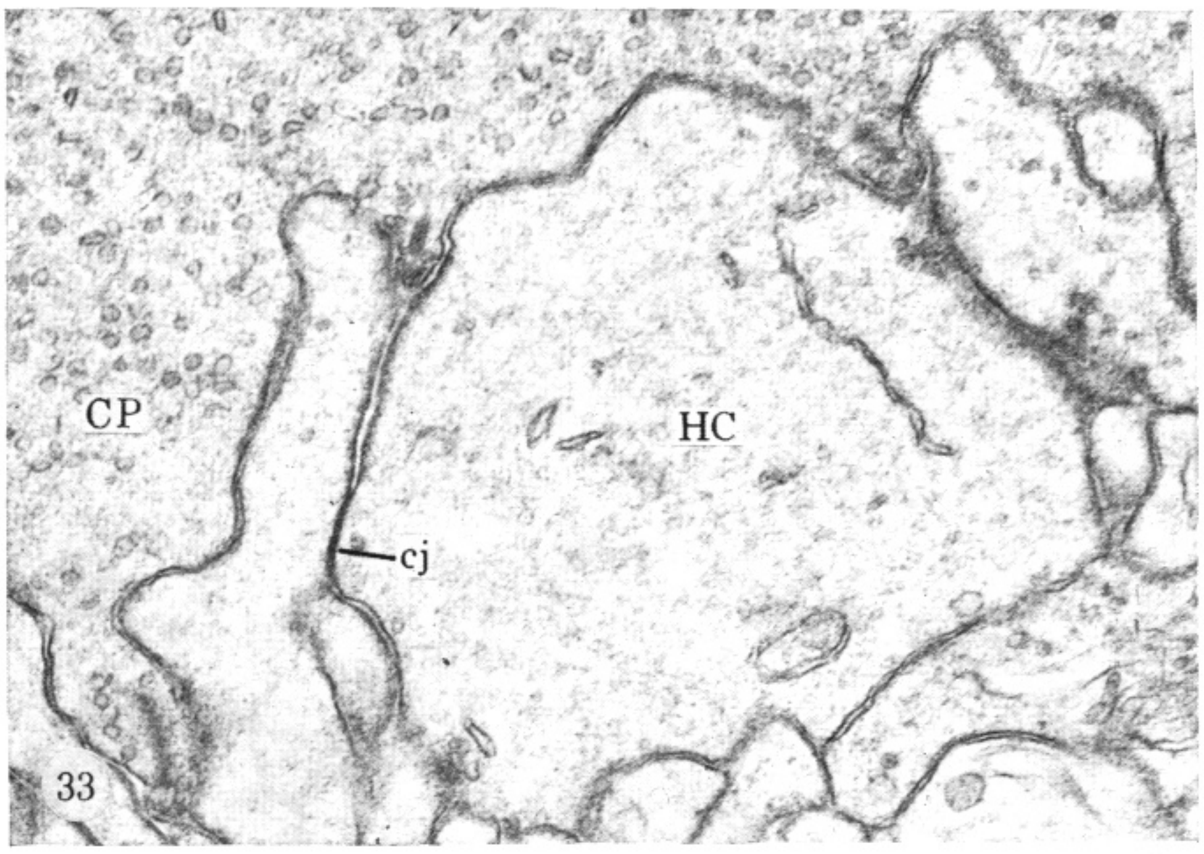

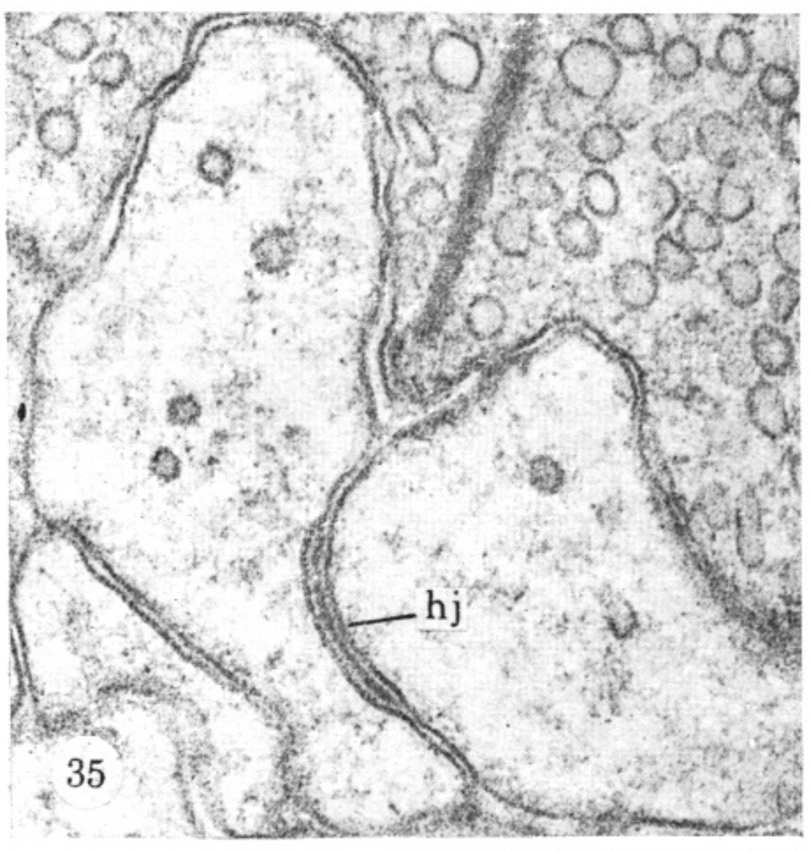

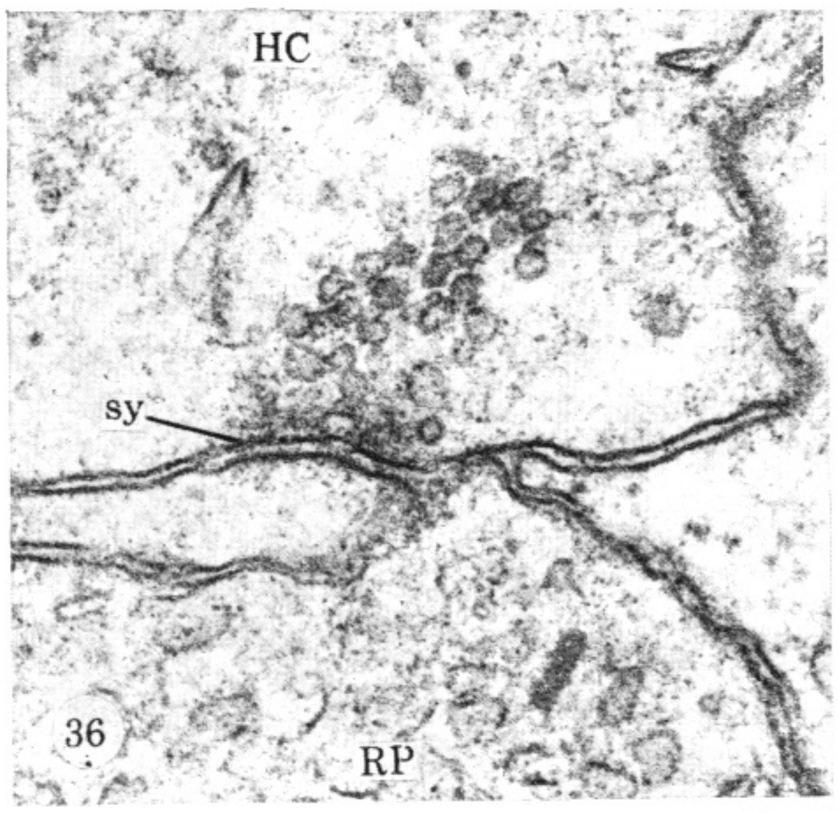

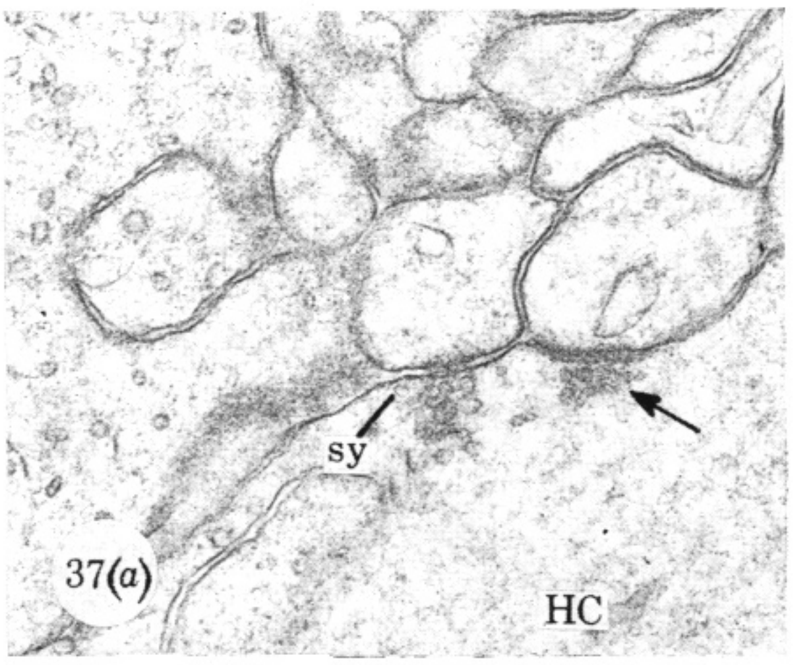

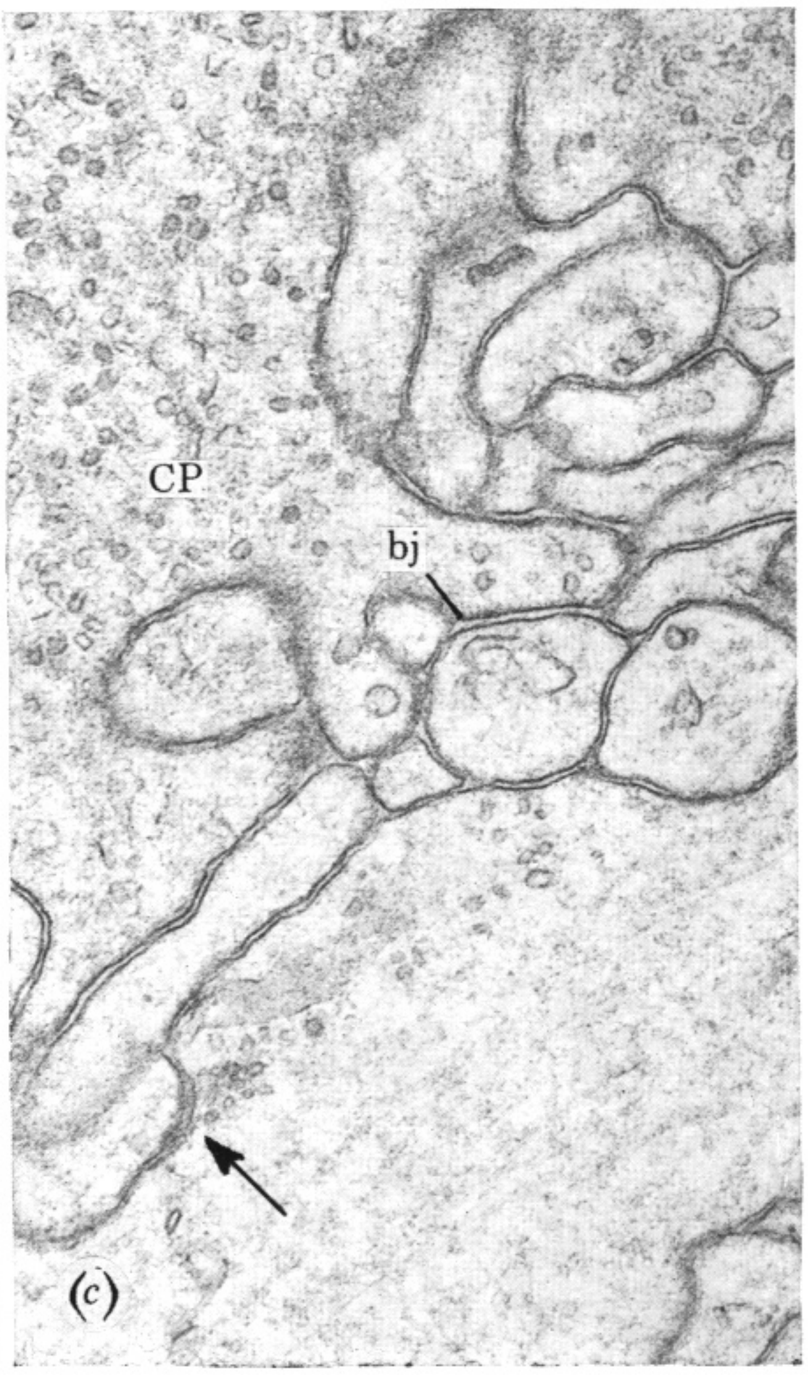

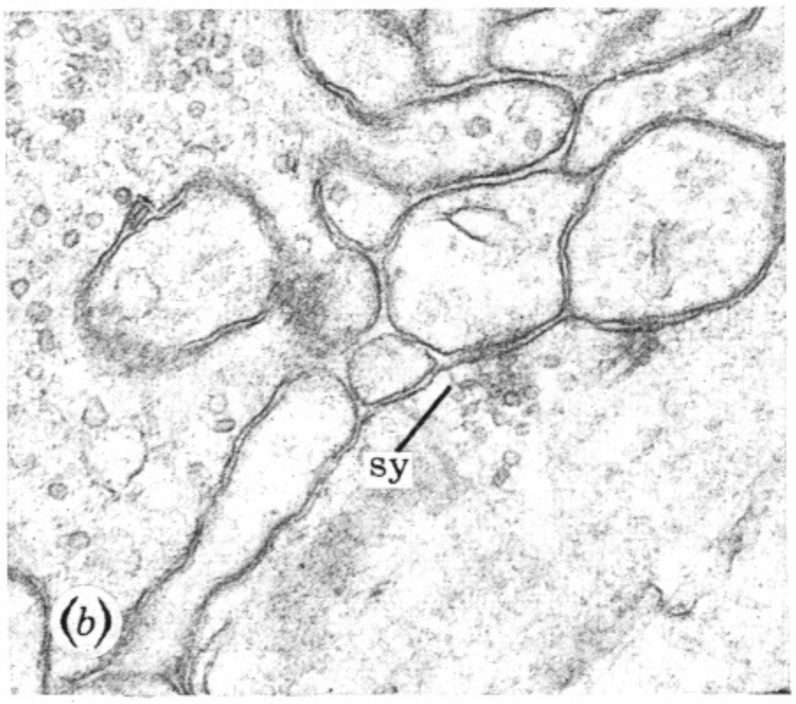

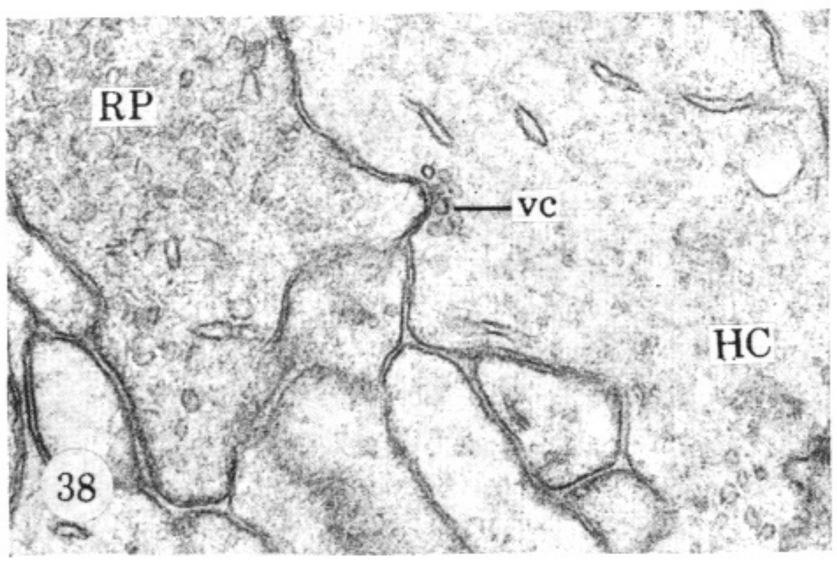

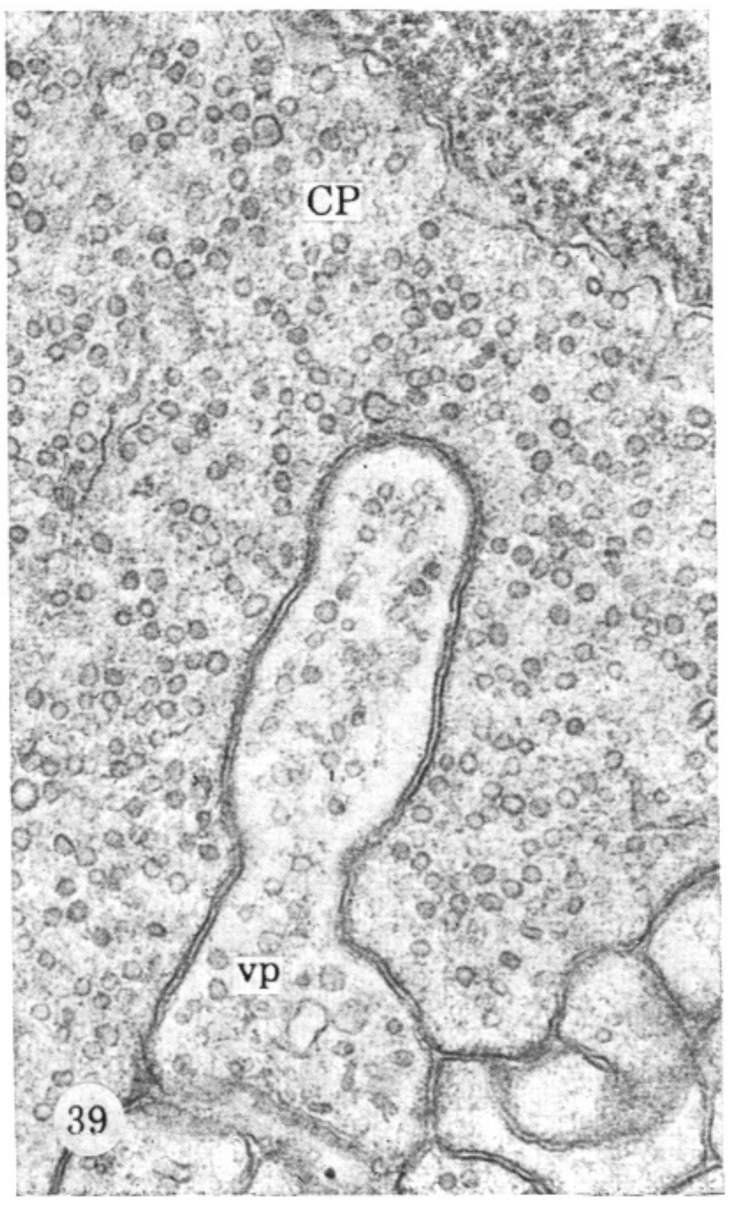

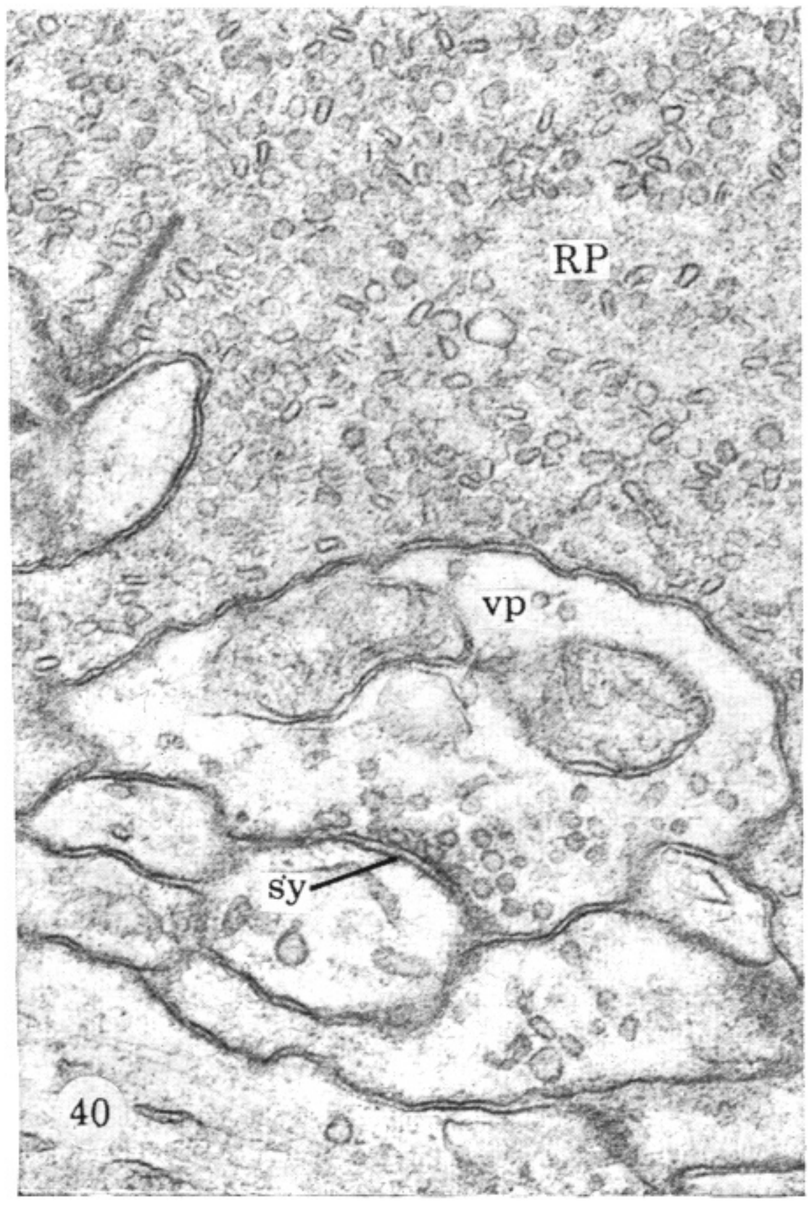

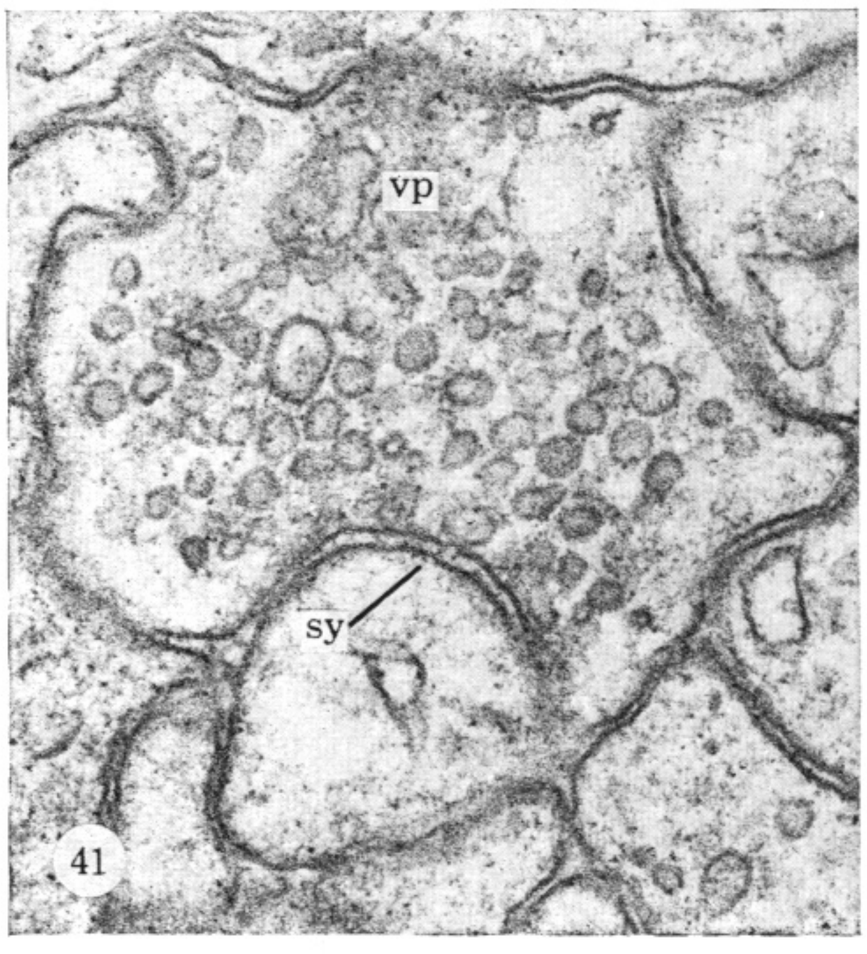

FIGURES 33 TO 41. For legends see facing page.