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ELECTRON MICROSCOPIC STUDIES ON THE EPENDYMAL CELLS OF THE ORGANON VASCULOSUM LAMINAE TERMINALIS IN THE ADULT RAT

BY

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ABSTRACT

The organon vasculosum laminae terminalis has been studied by many investigators in various animals.

Recently Weindl et al. ('67)[1] studied electron microscopically the organ of the rabbit, particularly the vascular system of the organ. Although there are many investigations on the organ, its function remains obscure. Therefore, in the present study the author attempted to investigate this organ electron microscopically with special reference to the ependymal cells.

The ependymal cells, unlike the ordinary ones, have neither cilia nor microvilli. Instead, two kinds of ependymal protrusions can be found, which is confirmed by making their three-dimensional reconstructions out of their serial electron micrographs. The *first type* is not a microvillus, but a fin-like thin fold of the apical cytoplasm akin to the *marginal fold* of Fawcett ('63, '65, '66)[2-4]. This type of protrusion has not been reported previously in the ependymal cells.

The *second type* is a protrusion of the kind that is irregular in shape and size, and in most cases, it shows *"coated"* and *uncoated invaginations* or *vesicles*. Moreover, in the apical cytoplasm are observed a large number of smooth-surfaced vesicles and some multivesicular bodies and also dense bodies. All these structures are considered to be closely related to absorption.

INTRODUCTION

Since Behnsen ('27)[5] first described the strong deposition of trypan blue in this brain area, this vascular organ, as one of the special regions without the blood-brain barrier, has been studied by many light microscopists.

Wislocki and his collaborators[6-8] studied the organ especially by vital staining and pointed out that the ependyma stains more deeply than the adjacent ependymal covering. Histochemically, Leduc and Wislocki ('52)[9], and Weindl ('65)[10] demonstrated the strong metabolic activity of this organ. Shimizu and Kumamoto ('52)[11], and Shimizu ('55)[12] also demonstrated a moderate deposition of glycogen within the ependymal cells.

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In this study, the author attempted to investigate the fine structure of the ependymal cells of this organ by electron microscopy.

Materials and Methods

Three adult male and female rats were employed in this study. Under anesthesia with 25 mg/kg Nembutal given intraperitoneally, the skull was opened at the parietal portion. Then a needle was inserted into each of the lateral ventricles to afford an outlet for the fixative, which was 2% osmium tetroxide, buffered at pH 7.4 with Veronal acetate[13] and containing 0.14 M sucrose[14]. Subsequently the fixative was injected very slowly through a needle into the third ventricle during a period of about ten minutes and simultaneously into the prechiasmatic cisterna as well. Immediately thereafter, the brain was carefully removed, immersed and cut paramedianly in fresh fixative as above. Favorably, the third ventricle was sharply outlined by osmium tetroxide and this organ was situated in the lamina terminalis just dorsal to the optic chiasm, evidently protruding into the optic recess in a crescent shape (Fig. 1). Immediately after it was diced under a binocular microscope into ca. 1.5 mm square blocks including the entire organ, they were immersed and fixed in the fresh fixative mentioned above for two additional hours, and then dehydrated in ascending concentrations of ethanol, and finally embedded in Epon 812[15]. Thin sections with gold to silver interference colors were cut with glass knives on a Porter-Blum microtome and stained with lead acetate[16] and then examined with a HS-7 electron microscope at original magnifications of 1,500 to 20,000 times. For light microscopy, adjacent thicker sections approximately 3 μ thick were stained with toluidine blue[17]. In addition, some three-dimensional reconstructions of the ependymal protrusions were made by gluing electron micrographs of four to six serial sections onto dental wax plates 4.5 mm in thickness (Figs. 6, 14, 16).

Observations

It seems interesting to study the fine structure of the ependymal cells covering the organon vasculosum laminae terminalis of the adult rat, since no one has paid attention to this subject.

i) Nucleus (Figs. 2, 4, 5, 20)

The nucleus is, on the whole, round or oval, but occasionally shows one or more indentations (Fig. 2; id). Its location in the cell, unlike that in the ordinary ependymal cell, is quite various (Fig. 2). The nucleolus is not so prominent, but sometimes it is located eccentrically near the nuclear

membrane (Fig. 4; no). Nuclear pores are relatively numerous (Fig. 5; arrows). Occasionally, the perinuclear space between the two membranes of the nuclear envelope is fairly distended into the perinuclear cisterna (Fig. 2; pnc). The outer nuclear membrane is only occasionally studded with ribosomes (Fig. 20; short arrow).

ii) Lateral cell surface (Figs. 2, 3, 4, 10)

Near the apices of adjacent ependymal cells, the *zonula* (or *fascia*) *occludens* and *zonula* (or *fascia*) *adhaerens*[18] are always present, but desmosomes can never be found. The *zonula* (or *fascia*) *occludens,* like the *zonula* (or *fascia*) *adhaerens,* is frequently encountered as the first luminal junction (Fig. 3). Just beneath these junctions the lateral cell membranes exhibit loose foldings (Figs. 2, 10; fo), and below these foldings they show no specialized intercellular junctions. Large intercellular or vacuolar spaces are rarely recognized between the ependymal cells, while nerve endings and interependymal plexus of unmyelinated nerve fibers are sometimes seen interposed between them (Figs. 4, 10). But, there is no evidence of synaptic contacts between the nerve fibers and ependymal cells.

iii) Basal cell surface (Figs. 2, 4, 10)

Unlike the adult mammalian ordinary ependyma, the basal surface is not always flattened and some cells are found to extend a single stout basal process into the underlying area (Fig. 4; bp). These cells are presumed to correspond to the so-called ependymal tanycyte first described by Horstmann ('54)[19]. But, it was not certain whether or not the basal process extends to the blood vessel or the pia mater. No basement membrane can be observed beneath the ependyma. The basal plasma membrane, like the lateral one, rarely forms invaginations showing the *caveolae intracellulares*[20]. Between the ependymal and the subjacent cell, neither special junctions nor synapses can be found, but only occasionally a somewhat dilated extracellular space can be found.

iv) Luminal cell surface

On the luminal surface, neither cilia nor microvilli can be found, but instead of them, two sorts of protrusions (the *first* and the *second type*) are recognized. Two kinds of surface specializations (*coated* and *uncoated invaginations*) are also visible in close association with them.

a) The *first type* of protrusion (thin fold type) (Figs. 4, 6–11, 13, 14; ft)

This is a fin-like, flattened fold (ca. 500 to 900 Å thick) of the apical cytoplasm. This type of protrusion has not yet been reported in the ependyma; however, on the free surface of the ependymal cells of this organ, this type of protrusion is occasionally encountered. It is at most 1 μ

long. In a single section, the protrusion has a finger-like or microvillus-like appearance (Figs. 7, 8, 13; ft). But, no circular profiles (ca. 500 to 900 Å in diameter) can be observed. So, a three-dimensional observation of these protrusions was made by making their reconstructions with the method mentioned above. As a result, it was verified that this type of protrusion is not a columnar microvillus but a palm-like or fin-like thin fold of the apical cytoplasm (Figs. 6, 14; ft). As may be seen in Fig. 9, which is probably an oblique or almost tangential section of this first type, their wavy appearance would suggest that these protrusions might be a "membrane ondulante" or "voile" of Policard ('64)[21]. Moreover, these protrusions occasionally exhibit a sequence of images that is considered to represent the possible stages of the light microscopical "pinocytosis"[2-4,22-24]. Vacuoles similar to the "pinocytosis vacuoles"[2-4] are occasionally observed (Fig. 4; pv). Although in rare cases the luminal surface of the protrusions appears to be coated with a downy material, the coating is not so remarkable as microvilli[25].

Their matrix is finely granular and of moderate density. In this matrix are observed only rarely small vesicles.

As compared with the second type, this type is rather few, but in some cases, it protrudes also from the lateral surface of the second type into the ventricular cavity (Figs. 9, 11, 13).

On the plasma membrane at the bases of these folds are often present pits or caveloae intracellulares about 350 to 550 Å in diameter (Figs. 8, 11).

b) The *second type* of protrusion (irregular bulbous type) (Figs. 2, 6, 7, 9–20, 23; st)

This is the type of protrusion that does not have a fixed shape and size, although the contour is on the whole roundish. These protrusions are more or less encountered on the apical surface of almost all the ependymal cells of this organ, and their thin sections are of various but roundish shapes: tongue-like, polypous, fungous, pseudopodial, cactoid, etc.; in some cases, as illustrated in Figs. 11–13, 18, 20, they present a very complicated appearance. Their reconstructions made with the same method as in the first type revealed that the second type is a protrusion of the apical cytoplasm that assumes extremely varied three-dimensional forms (Fig. 14, st). In Fig. 16, a racket-shaped projection is seen protruding with a slender stem from the luminal surface into the third ventricle, but in the apical cytoplasm just beneath the projection, no centriole can be detected. In Fig. 14, the first type of protrusions (ft) is observed protruding from the lateral surface of the second type, and the second type of protrusions (st) has an intricated appearance due to repeated constrictions. Therefore, the ventricular portion of the ependymal cell looks like a labyrinthic cave.

Moreover, just on the plasma membrane of the constricted parts, flask-shaped *"coated"* or *uncoated caveolae* (or *pits*) are observed (Figs. 11, 17–19).

Within the protrusions, some round or oval *"coated"* or *uncoated vesicles* and in some cases, free ribosomes or presumably glycogen granules are found (Figs. 11, 12, 17, rb; 23, gg), but the other organelles cannot be recognized.

The cytoplasmic surface of the *"coated" caveolae* (or *pits*)[3,4,26–28] is covered with a bristle-like coat of approximately 180 Å thickness, while their luminal surface is covered with a dense amorphous substance (Figs. 18, 19; cp).

"Coated" vesicles[3,4,26–28] (Fig. 15; cv_1), which can occasionally be found within these protrusions, are round or oval and about 650 to 1200 Å in diameter and have the same considerably great internal density and bristle-like coat as the coated caveolae (or pits). Similar vesicles are seen also in the apical cytoplasm as mentioned below.

Uncoated caveolae (or *pits*) (Figs. 11, 17; uc or up) are often seen exclusively on the luminal surface of the constricted parts of the second type as well as on that at the base of the first type of protrusion.

Uncoated vesicles (Figs. 11–13, 18, 20; uv) are also frequently found just adjacent to these invaginations within the second type of protrusion. These vesicles are for the most part round or oval and approximately 350 to 650 Å in diameter; their interior is of the same density as the uncoated caveolae (or pits), and mostly, of lower density than that of the coated. These vesicles are often seen in contact or confluent with each other (Figs. 11, 12). Similar but somewhat larger vesicles are also present in great numbers in the apical cytoplasm.

v) The cytoplasm

a) Mitochondria (Figs. 10, 20, 23; m): Though they are scattered throughout the cytoplasm, mitochondria are numerous especially in the supranuclear region and are sometimes found aggregated just below some of the protrusions. These mitochondria are round, oval, or rod-shaped in profile, but not ramified; their matrix is of homogeneous appreciable density and finely granular and, in most cases, contains a few round, dispersed, electron dense granules (about 300 to 400 Å in diameter) (Fig. 23; ig). Their cristae are oriented longitudinally or sometimes transversely.

b) Golgi complex (Fig. 10; gc): The Golgi complex is usually located in the supranuclear or paranuclear region, but rarely near the lateral cell membranes. This complex, unlike that of the ordinary ependymal cells, is relatively well developed and, in some cases, is found in two or three portions in the perikaryon. It consists of the following three kinds of elements: multilayered arrays of flattened cisternae with variable internal density, some large closely associated vacuoles, and numerous small vesicles. No granules can be seen within the cisternae and vacuoles.

c) Rough-surfaced endoplasmic reticulum (Figs. 12, 15, 20, 21, 23; rer):

Although the degree of its development varies considerably with the cells, this reticulum is in general not so well developed. The reticulum is composed chiefly of irregularly dilated cisternae, and the tubular elements can hardly be found in the cytoplasm. The cisternae are seen dispersed mainly in the apical or supranuclear cytoplasm and mingled with numerous smooth-surfaced vesicles and, in some places, directly connected with these vesicles (Fig. 21). The interior of the cisternae is of moderate density and in some cases, contains several fine granules (Figs. 12, 21, 23; g). The direct continuity of the reticulum with the Golgi complex, nuclear envelope, or plasma membrane is uncertain.

d) Smooth-surfaced endoplasmic reticulum (Figs. 5, 10, 15, 17, 20, 23; sv): Throughout the cytoplasm, there are a large number of smooth-surfaced vesicles. They are much more numerous than those in the ordinary ependymal cells. Tubular profiles can hardly be observed. These vesicles are usually spherical or ellipsoidal and of various sizes: their maximum diameter ranges from about 350 to 2000 Å. Accordingly, they are on the whole larger than the uncoated vesicles within the protrusions. Their internal density is also various and, occasionally, some of these vesicles have the same internal density as the coated ones (Figs. 23, 24). These vesicles are often seen adjoining or fusing with each other (Figs. 17, 23; thin arrow), but they are rarely found abutting on the lateral plasma membranes.

e) Coated vesicles (Figs. 15, 23, 24; cv): In the apical cytoplasm coated vesicles are sometimes found. Their shape, size, internal density, and surface specialization are similar to those of the coated ones within the protrusions: they are mostly round or oval, but in rare cases, crescent-shaped vesicles are also encountered (Fig. 23; thick arrow); they are about 650 to 1200 Å in maximum diameter. On rare occasions, similar vesicles are found also in the region of the Golgi complex, but are not observed in close association with the lateral or basal plasma membrane.

f) Multivesicular bodies (Figs. 10, 11, 20, 23; mb): Multivesicular bodies are also encountered often in the apical or Golgi area. They are commonly spherical and approximately 250 to 450 mμ in diameter, enclosing, in profile, ten to thirty round small vesicles about 300 to 450 Å in diameter. The internal density of these vesicles is slightly greater than that of the surrounding cytoplasm and their limiting membrane is not structurally specialized like that of these bodies.

g) Dense bodies (Figs. 11, 15, 20, 22; db): Limited by a single smooth-surfaced membrane, dense bodies are frequently seen in the apical or supranuclear cytoplasm. These bodies are usually round or oval, but in some cases, rod- or gourd-shaped. They are therefore various in dimensions (ca. 700 to 2000 Å in diameter by ca. 1000 to 5500 Å long). Within the bodies are dispersed many dense fine granules about 100 Å in diameter

(Fig. 22). In some cases, these bodies are found in contact or confluent with smooth-surfaced vesicles (Fig. 22; arrows).

h) Lipid droplets (Figs. 2, 4; ld): In the supranuclear region large lipid droplets are occasionally encountered. They are irregular in shape and in some cases, as large as more than 2 μ; they are usually devoid of a limiting membrane and their exceedingly dense interiors often display a stripe-pattern. Associated with these droplets no mitochondria can be found.

i) Filaments (Figs. 15, 20; f): A considerable number of cytoplasmic filaments about 90 Å in diameter are seen dispersed throughout the cytoplasm, but not so prominent as those in the ordinary ependymal cells.

j) Centrioles (Fig. 5; ce): Just beneath the luminal surface was rarely found a centriole, which is believed to be an anlage of a ciliary basal body. Besides, it was not found in close association with the two types of protrusions mentioned above.

k) Free ribosomes (Figs. 11, 12, 15, 20, 23; rb): Many free ribosomes ca. 150 Å in diameter are always seen scattered throughout the cytoplasm and frequently even within the protrusions of the second type. They are usually arranged in isolated clusters or rosettes and sometimes in a row.

l) Glycogen granules (Figs. 21, 23; gg): Coarse granules, presumably glycogen granules[29], are sometimes found dispersed in the apical cytoplasm and, occasionally, are found in clusters within the protrusions of the second type.

Discussion

Although many light microscopical studies on the organon vasculosum laminae terminalis have been made[5–12,30–39], there are no descriptions of electron microscopical findings on the ependymal cells of this organ. In all of the three adult rats examined, it was well developed and obviously located in the lamina terminalis just dorsal to the optic chiasm, protruding into the optic recess (Fig. 1). By light microscopy it is known that, in all species examined previously, the ependymal cells differ from the ordinary ones at the other sites of the ventricular system in shape and arrangement[8,10,30–39], which was also ascertained in this study by electron microscopy.

The luminal surface, as compared with that of the general ependyma[40–47], was rather flat and exhibited neither cilia nor microvilli, but showed two kinds of protrusions (the *first type* and the *second type*) and, in close association with them, two kinds of pits or caveolae (*coated* and *uncoated pits* or *caveolae*).

In sections, the *first type* of protrusion has an appearance like a microvillus. But the three-dimensional reconstructions, which were made

out of serial electron micrographs, revealed that these protrusions are not finger-like microvilli but fin-like folds (Figs. 6, 14; ft_1 and ft_2).

On the other hand, Brightman and Palay ('63)[45], dealing with the ependyma of the third and fourth ventricle and partly of the aqueduct of Sylvius, stated that these finger-like projections are too irregular in dimensions to be termed microvilli. But, three-dimensional studies with the reconstruction method have not been attempted on the ependymal protrusions before and fin-like protrusions like the *first type* of the author have not been reported in the ependyma. Therefore, it is probable that some of the ependymal projections described previously as microvilli or finger-like projections may be protrusions of this sort. Furthermore, this type of protrusion closely resembles the *marginal fold* of Fawcett ('63, '65, '66)[2-4]. Accordingly, the occurrence of this type of protrusion in this organ may suggest the potentiality of *pinocytosis*[2-4,22-24] of the ependymal cells covering this organ.

Moreover, on the plasma membrane at the bases of these protrusions are frequently seen pits or caveolae. These invaginations probably suggest *micropinocytosis*[3,4,50,51] of the ependymal cells.

On the other hand, the *second type* (*irregular bulbous type*) is a protrusion of the apical cytoplasm that may have extremely various three-dimensional shapes and sizes, which was ascertained by the reconstruction method mentioned above. This second type of protrusions shows many constrictions, where coated or uncoated invaginations are often seen. Within these protrusions, coated or uncoated vesicles, free ribosomes and sometimes glycogen granules are found, but no other organelles can be recognized. Consequently, this second type is markedly different from the first type and also from regular microvilli. Furthermore, even in profiles, they can be distinguished from intraventricular nerve endings since the latter is reported to contain some other organelles (mitochondria, cored vesicles, microtubules, etc.)[45,48,49]. In addition, since no anlage of the ciliary basal body can be detected in close association with these protrusions, they are not the incipient or immature cilia of Sotelo et al. ('58)[52], and Tennyson and Pappas ('62)[44]. They are presumably transient protrusions which may be formed due to the increased surface activity.

Moreover, Brightman ('65a, b, c, '66)[53-56] has shown in the ependymal cells that ferritin, when injected intraventricularly, is ingested via the plasma membrane invaginations into the coated and uncoated vesicles and then segregated within the vacuoles, multivesicular bodies, and dense bodies. Therefore, these structures may also suggest the absorptive function[28,57] of the ependyma of this organ.

Concerning the interependymal junctions of the rat, there are the detailed descriptions by Brightman and Palay ('63)[45], and Brightman

('65a)[53], which appear almost in accordance with the author's. But, in this organ, the *zonula* or *fascia occludens* was also frequently encountered as the first luminal junction (Fig. 3).

Finally, in the ependymal cells covering this organ are observed well developed Golgi complex, lipid droplets and presumably glycogen granules. These structures seem to have some relationship to the special functional state of the ependyma of this organ.

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Explanation of Plates

Plate I

Fig. 1. Photomicrograph of a paramedian sagittal section through the *organon vasculosum laminae terminalis* (ov) of an adult rat. Note that the organ is located in the lamina terminalis (lt) just dorsal to the optic chiasm (oc) protruding into the optic recess (or). ca: Commissura anterior. H.E. ×15.

Fig. 2. Low power electron micrograph of a part of the ventricular wall of this organ. The ependymal cells (ep) exhibit considerable multiformity and irregularity in shape and arrangement, and have neither cilia nor microvilli. At arrows, protrusions of the *second type* (st: see text) are observed. The nuclei (n) show an occasional indentation (id), nuclear pores (np), and perinuclear cisternae (pnc). The lateral plasma membranes are loosely infolded (fo). gc: Golgi complex; ld: lipid droplet. ×4000.

Fig. 3. Electron micrograph showing interependymal junctions: the *zonula occludens* (zo_1 and zo_2) and the *zonula adhaerens* (za). Note that the *zonula occludens* (zo_1) is present also as the first luminal junction. app: simple apposition; m: mitochondrium. ×41300.

Fig. 4. Low power electron micrograph of three ependymal cells (ep). To the upper left, a protrusion of the *first type* (ft: see text) and a vacuole similar to the "pinocytosis vacuole" (pv) can be seen. The middle ependymal cell contains a large lipid droplet (ld) and extends a stout basal process (bp) into the underlying tissue. A poorly developed nucleolus (no) is seen located eccentrically near the nuclear envelope. Unmyelinated nerve fibers (un) are observed interor subependymally. gc: Golgi complex. ×4800.

Fig. 5. Electron micrograph of an ependymal cell showing many nuclear pores (arrows), numerous smooth-surfaced vesicles (sv), and a centriole (ce). ×10700.

Figs. 6 and 7. Fig. 6 is a stereo view of a three-dimensional wax plate reconstruction of ependymal luminal protrusions based on six serial electron micrographs, one of which is illustrated in Fig. 7. In a single section, protrusions of the *first type* have a finger-like or microvillus-like appearance (ft_1 and ft_2 in Fig. 7), but the reconstruction reveals that the protrusions are not microvilli but palm-like or fin-like thin folds of the apical cytoplasm (ft_1 and ft_2 in Fig. 6). Three protrusions of the *second type* are also visible (st_1, st_2, and st_3 in Fig. 6). ap: apical portion of ependymal cell. One side of the lower left cube in Fig. 6 represents 0.2 μ. Fig. 7: ×13200.

Plate II

Fig. 8. Electron micrograph of two protrusions of the *first type* (ft) showing an uncoated pit (up) on the plasma membrane at their bases. ap: apical portion of ependymal cell. ×41200.

Fig. 9. In this electron micrograph, a few protrusions of the first type in oblique or almost tangential section are illustrated (ft_1, ft_2, ft_3, and ft_4). Note that they present a palm-like and wavy appearance, and that at the arrow, the tip of the protrusion is in close contact with the luminal plasma membrane. n: nucleus of ependymal cell; st: the second type of protrusion. ×22500.

Fig. 10. Survey electron micrograph of an ependymal cell showing many irregularly-shaped protrusions on the luminal surface. No cilia can be detected. Note many supranuclear mitochondria (m) of appreciable internal density, well developed Golgi complex (gc_1, gc_2, and gc_3), multivesicular bodies (mb_1 and mb_2), dense bodies (db), and numerous smooth-surfaced vesicles (sv). Loose foldings (fo) of the lateral plasma membranes, interependymal plexus of unmyelinated nerve fibers (iep), and an occasional nerve ending (ne) are also visible, but no basement membrane can be recognized. See also Fig. 11. ×9400.

Fig. 11. Higher magnification of the indicated area in Fig. 10 to show *uncoated caveolae* (uc), a multivesicular body (mb), and a rod-shaped dense body (db). Note the uncoated invaginations (uc_1–uc_4) on the luminal plasma membrane of the *second type* of protrusion (st) as well as on that at the bases of the *first type* of protrusions (ft_2 and ft_3); just adjacent to these invaginations, uncoated vesicles (uv) are also present, and at arrows, these vesicles are seen in contact or confluent with each other. The multivesicular body is of moderate matrix density and encloses many small vesicles, some of which are seen adjoining each other. rb: free ribosome. ×36800.

Fig. 12. Electron micrograph of irregularly-shaped protrusions of the *second type* (st) showing numerous free ribosomes (rb) and uncoated vesicles (uv). Some uncoated vesicles are seen just adjacent to the invagination of the plasma membrane (arrow). The rough-surfaced endoplasmic reticulum (rer) is of moderate internal density and contains several fine granules (g). ×32500.

Plate III

Figs. 13 and 14. Fig. 14 is a stereo view of a three-dimensional wax plate reconstruction of luminal protrusions based on four serial electron micrographs, one of which is illustrated partly in Fig. 13. It is well shown that the *first type* of protrusions (ft in Fig. 14) is a flattened thin fold of the apical cytoplasm (ac), and that the *second type* of protrusions (st in Fig. 14) assumes extremely varied three-dimensional forms. Within the second type of protrusions, many uncoated vesicles (uv in Fig. 13) are present. One side of the lower right cube in Fig. 14 represents 0.2 μ. Fig. 13: ×48800.

Fig. 15. Electron micrograph of a racket-shaped protrusion of the second type (st) showing a *coated vesicle* (cv_1) within it. In the apical cytoplasm, note many smooth-surfaced vesicles (sv), the sparse rough-surfaced endoplasmic reticulum (rer), numerous free ribosomes (rb), two dense bodies (db), a *coated vesicle* (cv_2), and many filaments (f). m: mitochondrium. ×32800.

Fig. 16. Stereo view of a three-dimensional reconstruction of the racket-shaped protrusion illustrated as st in Fig. 15. ap: apical portion of ependymal cell. One side of the lower right cube represents 0.2 μ.

Fig. 17. High power electron micrograph of a luminal protrusion of the second type (st) showing a tunnel-like uncoated pit (up) and many free ribosomes (rb). Smooth-surfaced vesicles (sv) are seen adjoining each other (thin arrow). m: mitochondrium. ×58900.

Fig. 18. Electron micrograph of an intricated protrusion of the second type (st)

showing a *coated pit* (arrow: see also Fig. 19). Within the protrusion, uncoated vesicles (uv) are also present. ep: ependymal cell. ×25400.

Fig. 19. Higher magnification of the rectangular area in Fig. 18 to show details of the *coated pit* (cp). Note that the pit demonstrates a felt- or bristle-like coat on the cytoplasmic surface, and a dense amorphous substance in the cavity. uv: uncoated vesicle. ×100000.

Plate IV

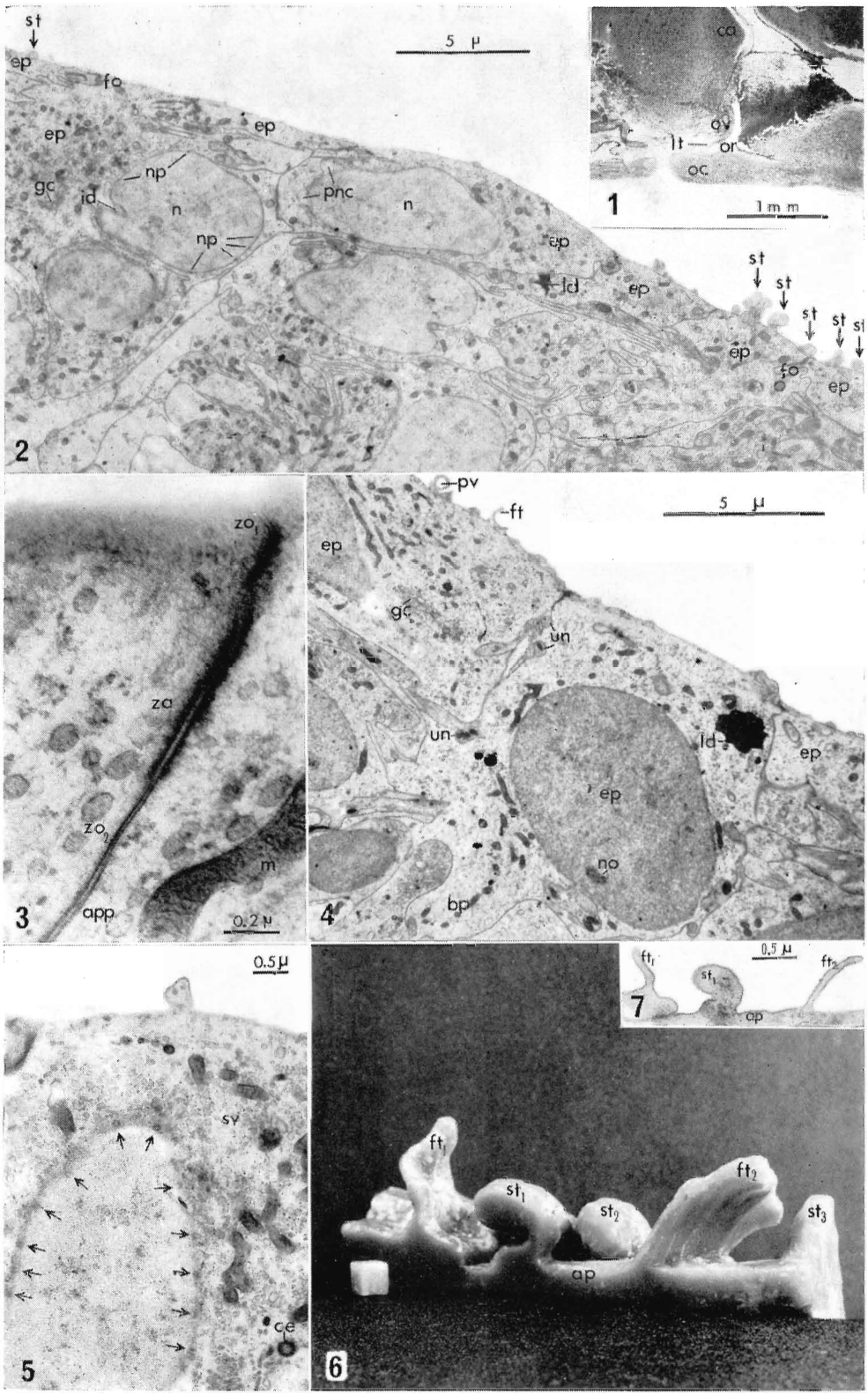
Fig. 20. Electron micrograph of a portion of an ependymal cell showing some irregularly-shaped protrusions (st). Note many smooth-surfaced vesicles (sv), dense bodies (db), filaments (f), mitochondria (m), and a multivesicular body (mb) delimited by a single membrane. The outer nuclear membrane is only sparsely studded with ribosomes (short arrow); at long arrows, nuclear pores (np) can be seen. ig: intramitochondrial granule; n: nucleus; rb: free ribosome; rer: rough-surfaced endoplasmic reticulum; uv: uncoated vesicle. ×14600.

Fig. 21. In this electron micrograph, a smooth-surfaced vesicle (sv) is seen confluent with the rough-surfaced endoplasmic reticulum (rer), within which some fine granules (g) are seen. gg: presumed glycogen granule. ×89400.

Fig. 22. High power electron micrograph of the supranuclear cytoplasm showing three *dense bodies* (db). Limited by a single membrane, the bodies contain many dense fine granules. At the arrow, one of the bodies is seen also in close contact with a smooth-surfaced vesicle (sv). m: mitochondrium. ×66000.

Fig. 23. Electron micrograph of an apical portion of an ependymal cell showing many *coated vesicles* (cv), numerous smooth-surfaced vesicles (sv) of variable size and density, a multivesicular body (mb), and many mitochondria (m) with some intramitochondrial granules (ig). The rough-surfaced endoplasmic reticulum (rer) is seen containing fine granules (g). Note the protrusion (st) crowded with presumed glycogen granules (gg), which can be seen dispersed also in the apical cytoplasm. thin arrows: smooth-surfaced vesicles fusing with each other; thick arrow: a crescent-shaped coated vesicle. ×46000.

Fig. 24. Higher magnification of the indicated area in Fig. 23 to show details of the *coated vesicle* (cv). Note the felt- or bristle-like coat on the cytoplasmic surface (arrows) and the dense amorphous substance in the interior. The lower left vesicle, though devoid of the bristle coat, is of the similar internal density and size to those of the coated one. ×140000.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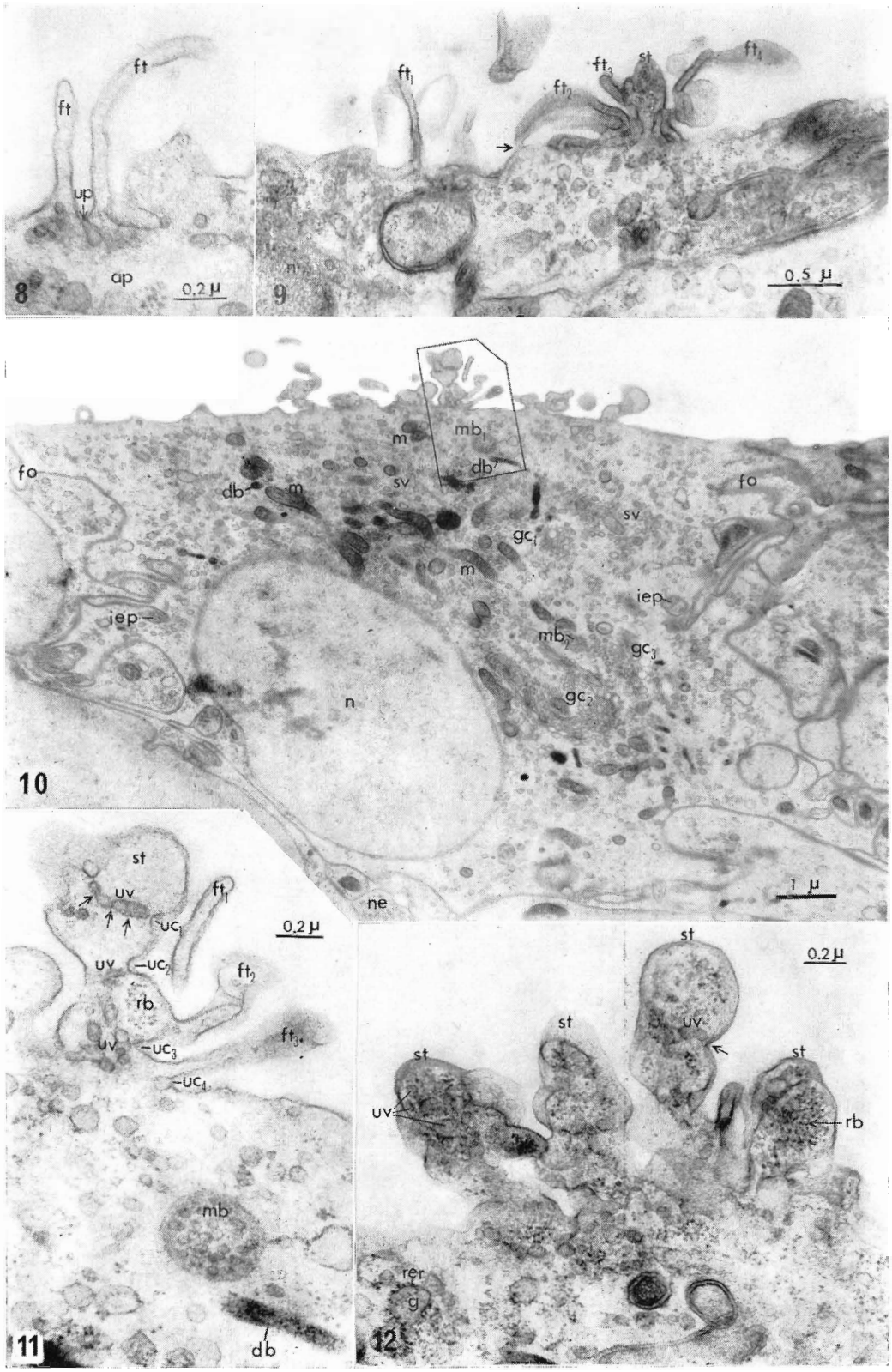
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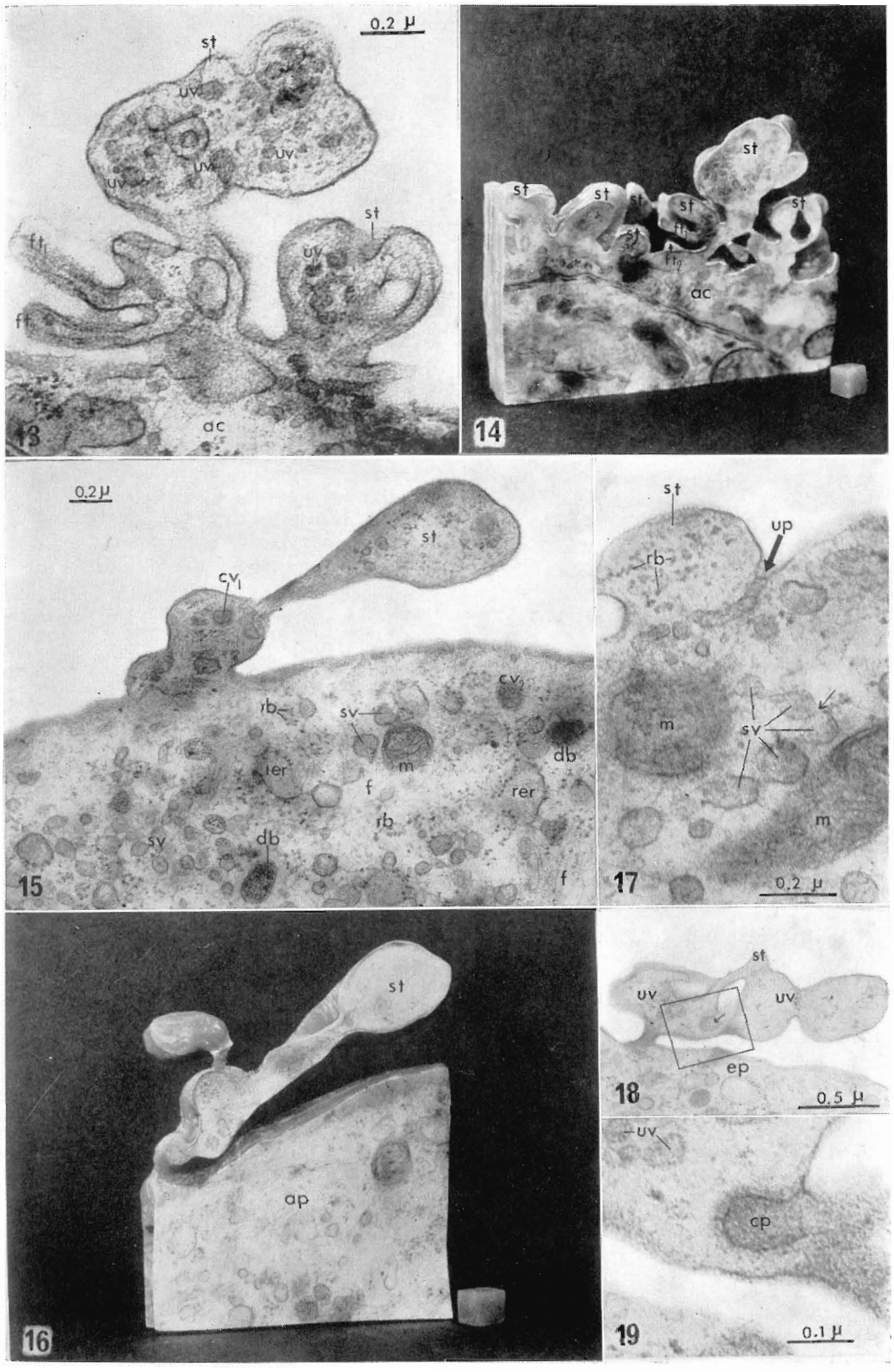
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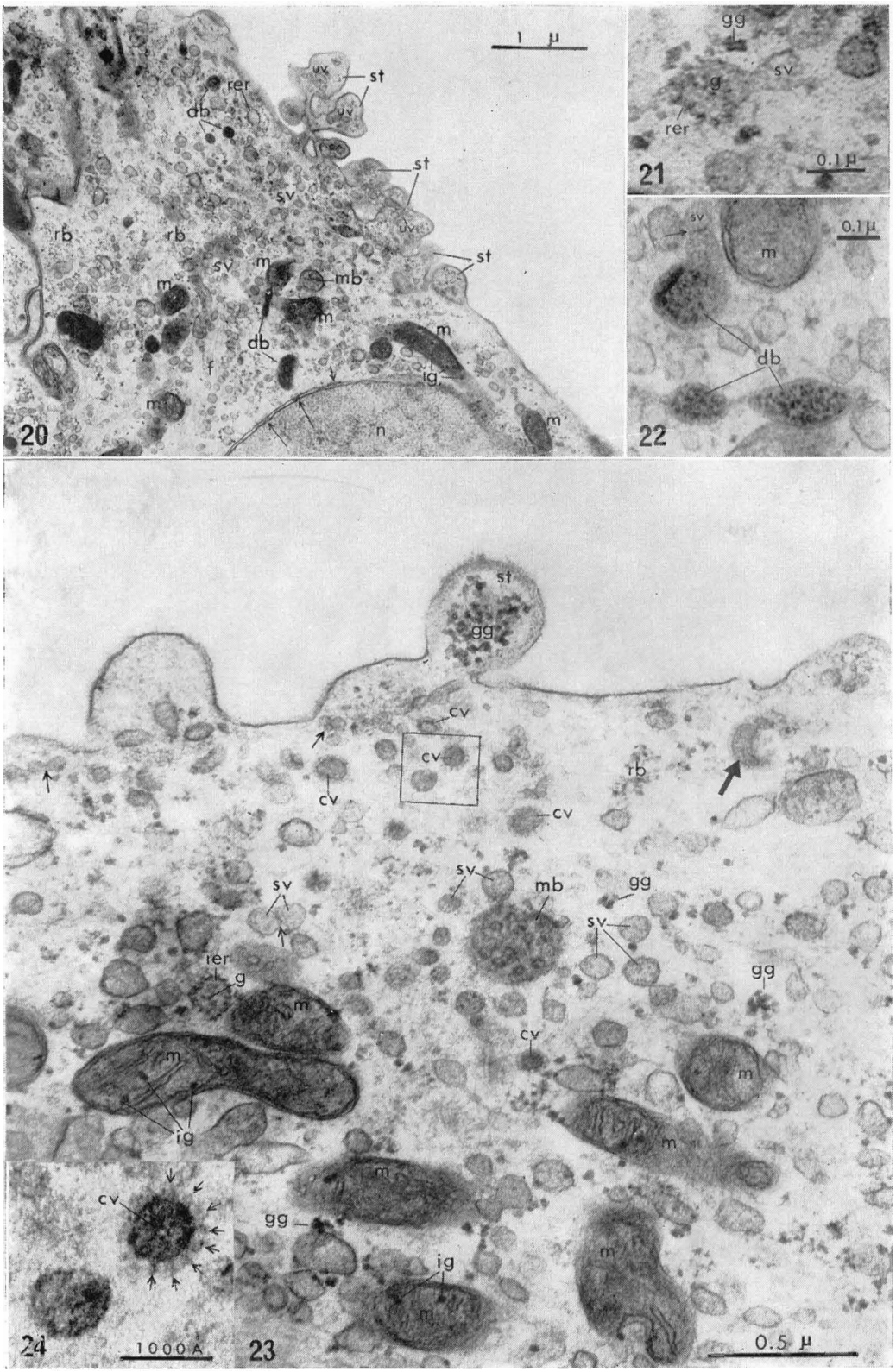
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