Scanning and Transmission Electron Microscopy of the Ependymal Lining of the Third Ventricle

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SUMMARY: In its simplest form, the ependyma of the third ventricle consists of a single layer of cuboidal cells. Although these typical mural cells constitute the greater part of the lining of the ventricle, a specialized variety of ependymal cell (the tanycyte) can also be distinguished within circumscribed areas of the ventricular wall. Although such cells are found scattered throughout the dorsoventral extent of the third ventricle, they are particularly numerous along the ventrolateral walls and floor. The regional variation in the surface morphology of the ventricle walls as evident with the scanning electron microscope is consistent with this pattern of tanycyte distribution. Ultrastructural studies have established that the tanycyte is a fundamentally distinct cell with a long basal process extending into the subjacent neuropil and frequently directed toward a capillary wall. This unique morphology conforms closely to its three-dimensional appearance as demonstrated with the scanning electron microscope. The significance of ependymal tanycytes particularly of the third ventricle derives largely from the connections they establish between the ventricular lumen and vasculature of the median eminence. This intriguing structural relationship has led to the suggestion that ependymal cells and cerebrospinal fluid (CSF) may be involved in the regulation of adenohypophysial activity. Evidence indicating the functional involvement of specialized ependymal cells in the neuroendocrine control of pituitary activity is reviewed.

RESUME: L’épendyme du troisième ventricule est constitué essentiellement d’une seule couche de cellules cuboïdes. Bien que ces cellules murales typiques forment la plupart de l’enveloppe ventriculaire, on peut reconnaître cependant une variété spéciale de cellules épendymaires (la tanycyte) à de régions circonscrites de la paroi ventriculaire. De plus-celles-ci, quoi qu’elles soient distribuées de part et d’autre à travers l’entière entièrée dorso-ventrale du troisième ventricule, sont surtout nombreuses à ses régions ventro-latérales ainsi que son plancher. La variation morphologique régionale des parois ventriculaires observée avec le microscope électronique de balayage est d’accord avec une telle distribution de tanycytes. D’études ultrastructurales ont établi que la tanycyte soit bien une cellule essentiellement distinue avec une elongation basale rejoignant le neuropil sous-jacent et fréquemment dirigé vers une paroi capillaire. Cette morphologie unique conforme bien à son apparente en trois dimensions au microscope électronique de balayage. L’importance de tanycytes épendymaires du troisième ventricule survient surtout des interconnexions qu’elles établissent entre le troisième ventriculaire même et la vasculature de l’éminence médiane. Cette dernière relation structurelle a donné origine à la suggestion que les cellules épendymaires ainsi que le liquide céphalorachidien du troisième ventricule puissent participer au contrôle d’activité adenohypophysaire. Evidence de participation fonctionnelle de cellules épendymaires spécialisées au contrôle neuroendocrinien d’activité pituitaire est ici discuté.

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1. Introduction: Purkinje (1836) is credited with being the first to document the character of the epithelial lining of the cerebral ventricles. The introduction of metal impregnation techniques some years later awakened renewed interest in the subject and much new information concerning the morphology of the ependymal cell was soon obtained. Reviews of the earliest literature on this subject Studnicka (1900), Agduhr (1932), Wislocki (1932), indicate that the early investigators recognized the wide variation in ependymal cell morphology, and in particular, that some cells have long basal extensions incorporated into the brain parenchyma. There was little consensus in the terminology applied to these extensions until Horstmann (1954) introduced the descriptive term — tanycyte — for the elongated ependymal cells of Selachians that stained with the Cajal gold sublimate method. They were distinguishable from common mural ependyma by virtue of their long basal processes which extend into the subjacent neuropil. In recent years, the specialized ependymal cells situated along the floor and lateral walls of the infundibular recess (IR) have received particular attention because of the morphological link they establish between the cerebrospinal fluid (CSF) on the one hand, and the pars tuberalis and the vasculature of the median eminence (ME) on the other. This intriguing structural relationship led Lofgren (1959) (1960), to initially suggest a possible involvement in the neuroendocrine control of pituitary function. Although much inferential and speculative evidence of such an involvement has been subsequently
adduced, their precise nature and function is far from clear and merits further investigation.

2. Principal Cell Types in the Ventricular Wall: Distribution and Ultrastructure

Light and electron microscopy have been used to elucidate the fine structural details of the cerebral ventricular system in a number of species. Regional differences in the structure of both the ependyma and subependymal layers of the ventricles have been observed Fleischhauer (1961), Klinkerfuss (1964), Schimrigk (1966), Tennyson and Pappas (1968). The walls of the ventricles are generally lined with a continuous epithelium that varies from flattened squamous-like to cuboidal-columnar, with and without cilia and cell processes. Variations, however, in stratification and morphology occur with age, species, sex and location within the ventricular walls.

Several ependymal cell variants classified according to their morphology have been described Tennyson and Pappas (1962) Knowles and Anand Kumar (1969), Anand Kumar (1972), Knowles (1972), Millhouse (1972), Sharp (1972). In its simplest form, the ependyma of the rabbit third ventricle consists of a single layer of cuboidal cells (Fig. 1). The typical mural ependymal cell comprises the greater part of the lining of the third ventricle (re: section 4), and is, with minor exceptions, fundamentally similar to the characteristic ependyma described in other mammalian species Brightman and Palay (1963), Klinkerfuss (1964), Malinsky (1968), Knowles and Anand Kumar (1969), Westergaard (1970), Millhouse (1972). The luminal plasma membrane is complexly organized into numerous microvilli-like projections approximately 0.04-0.07 \( \mu m \) in diameter and tufts of cilia (Fig. 2). Projections of the juxtaventricular surface may contain small pits and vesicles, however cytoplasmic organelles are rarely included. Cilia are of indeterminate length, approximately 0.22 \( \mu m \) in diameter, and have the usual inner arrangement of 9 peripheral double-
endoplasmic reticulum. The Golgi complex, with its typical complement of vesicles and cisternae generally occupies a supranuclear position (Fig. 2). Randomly dispersed cytoplasmic filaments and microtubules were frequently observed, however dense perinuclear bundles of filaments as demonstrated in the rat Brightman and Palay (1963), Millhouse (1972), were not evident. Membrane bound multivesicular bodies and lysosomes are also common.

The lateral surfaces of adjacent ependymal cells are without elaborate folds and interdigitations. A detailed report of the complex intercellular junctions between contiguous ependymal cells in the rat has been published Brightman and Palay (1963). Our observations are in substantial agreement with these findings. The principal types of junctional complexes encountered in the rabbit were zonulae adhaerentes and ocludentes (Fig. 2). Typical desmosomes do not occur between ependymal cells. The basal surfaces of the ependymal cells are without elaborate plication or processes and lie directly on the underlying neuropil without an interposed basement membrane between them.

The ciliated cuboidal ependymal cells have been attributed various functions. Histochemical observations indicate that these are cells of high metabolic activity related to secretion, absorption or transport of substances from the vasculature into the ventricle or vice versa Nandy and Bourne (1964). In contrast, the enzymatic pattern has also been demonstrated using a variety of histological and histochemical procedures Fleischhauer (1961), Anand Kumar and Knowles (1967), Bleier (1972), Sharp (1972). Ultrastructural studies have established that the tanyctye is a fundamentally distinct cell with a single dense peripheral process that is generally unbranched except at its termination, corresponding to LM descriptions Tennyson and Pappas (1962), Leonhardt (1966), Brawer (1972), Millhouse (1972). Although clusters of such cells may be found scattered throughout the dorsoventral extent of the third ventricle, they are particularly numerous along the lateroventral walls and floor Knowles and Anand Kumar (1969), Millhouse (1971), Sharp (1972).

In addition to the typical ependymal cell, a second cell type, the ependymal astrocyte Horstmann (1954), has been recognized as a component of the ependymal wall. Tennyson and Pappas (1962), identified it as a columnar cell with a branching peripheral process of light density within the ependymal lining of the fetal rabbit midbrain. A sparsecy of mitochondria, endoplasmic reticulum and filaments, complex plication of the lateral cell membrane, and multiple thin projections from the cell body and main process, were cited as its distinguishing features. In the feline lateral ventricle, the presence of a similar cell was ascribed to the subependyma Klinkerfuss (1964). The ependymal nature of these cells therefore is open to question. Similarly, "glandular" cells distinguished among the ependyma in the anterior tuber cinereum of the Rhesus monkey Knowles and Anand Kumar (1969), Anand Kumar (1972), Knowles (1972), cannot be categorized with certainty. They may correspond to the specialized ependyma described in the IR of the rat, Lévéque and Hofkin (1961), Lévéque, Stutinsky, Porte and Stoeckel (1966), and the ventral "glandular" ependyma and/or type 4 tanyctyes of the quail, Sharp (1972). There are indications, however, that these cells may subserv a secretary capacity related to pituitary function.

The other principal cell variant distinguished within circumscribed areas of the ventricular wall is the ependymal tanyctye. Their presence has been demonstrated using a variety of histological and histochemical procedures Fleischhauer (1961), Anand Kumar and Knowles (1967), Bleier (1972), Sharp (1972). Ultrastructural studies have established that the tanyctye is a fundamentally distinct cell with a single dense peripheral process that is generally unbranched except at its termination, corresponding to LM descriptions Tennyson and Pappas (1962), Leonhardt (1966), Brawer (1972), Millhouse (1972). Although clusters of such cells may be found scattered throughout the dorsoventral extent of the third ventricle, they are particularly numerous along the lateroventral walls and floor Knowles and Anand Kumar (1969), Millhouse (1971), Sharp (1972).

An electron micrograph of an ependymal tanyctye soma and the proximal part of its long irregular process is shown in Fig. 3. Tanyctyes at this level of the rabbit third ventricle (along the lateral wall and floor of the IR) are furnished with very few cilia; rather their apical surfaces are characterized by an extensive elaboration of pleomorphic microvilli-like processes. These findings are in accord with both earlier ultrastructural reports Matsui and Kobayashi (1968), Knowles and Anand Kumar (1969), Brawer (1972), and recent SEM observations (re: sections 3, 4) in a number of species. The apical lateral membranes between adjacent cells may or may not be interdigitated. They generally contain intercellular junctional complexes similar to those described in common mural ependyma. A paucity of occluding junctions occur however, between tanyctyes in the arcuate region of the rat, Brawer (1972). The nuclei of rabbit tanyctyes are elongate and often deeply indented with one or more nucleoli. The somata contain the normal complement of organelles and inclusions some of which extend into the proximal and distal portions of their processes. Dispersed within the cytoplasm are numerous ribosomes, dilated cisternae of smooth endoplasmic reticulum and isolated profiles of granular reticulum. Mitochondria are numerous, particularly in the basal cytoplasm, and throughout the processes. The Golgi complex is prominent and situated either supra or infranuclearly. Vesicles, multivesicular bodies and lysosomes are common features of the soma as are microtubules and filaments. Millhouse (1972), cited nuclear shape, matrix density and the absence of bands of filaments surrounding the nucleus as the principal criteria for distinguishing tanyctyes in the rat. On the other hand, the cytology of rabbit ependymal cells was observed to be sufficiently similar to suggest that differentiation of cell types be determined by the morphology of their bases Tennyson and Pappas (1962).

The peripheral process of the tanyctye extends into the subjacent neuropil and is frequently directed toward a capillary wall. It characteristically has a dense appearance and contains numerous longitudinally oriented mitochondria, microtubules and filaments (Fig. 3, inset). Dilated cisternae and rosettes
of ribosomes are also present. In the postnatal rabbit, the number of organelles were observed to diminish distally in the tanyocyte processes Tennyson and Pappas (1962). In the rat, however, the small size and orientation of the mitochondria and the numerous highly oriented microtubules are diagnostic criteria for identifying tanyocyte processes in the neuropil. Brawer (1972). There is no indication in the rabbit, as in the rat, Millhouse (1972), that the basal processes of tanyocytes repeatedly branch to form a syncytium, Knowles and Anand Kumar (1969).

Although the cytological features of these cells and their relationship with other tissue components have been described in several reports, the criteria for identifying their terminal processes and distinguishing them from other processes in the neuropil remains uncertain. The tanyocyte processes is unbranched throughout its course, except at its termination where it divides into several slender branches. A pericapillary ependymal ending abutting directly on the basement membrane of a capillary perivascular space is shown in Fig. 4. These tanyocyte foot processes are reported to contain numerous microtubules and filaments, paraxially-oriented mitochondria, vesicles and granules.

Although the precise function of tanyocytes is far from clear, the literature is replete with conjecture, that will not be enumerated here. For a detailed discussion of several possibilities that have been advanced, the reader is referred to the work of Millhouse (1971), (1972). Indeed, recent morphological and cytochemical observations has even indicated structural and possibly functional differences among tanyocytes themselves Luppa and Feustel (1971), Millhouse (1971). Sharp (1972). Only the available evidence relating to the possible neuroendocrine function of those tanyocytes which contact the portal vasculature will be presently reviewed (re: section 6).

3. Structure of the Tanyocyte and its Vascular Termination:
Scanning Electron Microscopy

Although the scanning electron microscopy of the third ventricle

**Figure 3.** Transmission electron micrograph of an ependymal tanyocyte soma (Ts) and the proximal part of its basal prolongation (Tp) along the ventrolateral wall of the rabbit IR. (Nu), tanyocyte nucleus. X 9,200

Inset, basal process of the tanyocyte containing microtubules and filaments, mitochondria, ribosomes and smooth endoplasmic reticulum. X 20,000

**Figure 4.** Median eminence (external layer) illustrating the direct apposition of a tanyocyte foot-process (Tfp) on the basement membrane of a capillary (C) perivascular space. X 13,600

**Figure 5.** Scanning electron micrographs arranged as a montage illustrating the three dimensional structure of an ependymal tanyocyte at the level of the rabbit IR. The luminal surface of the soma (Ts) is nonciliated and its single tapering process (Tp) is unbranched throughout its course through the ME except at its termination where multiple endfeet (Tfp) are given off. (C), capillary (9 diam.). X 2,700
microscope (SEM) has been employed in morphological investigations of ependyma lining the mammalian cerebral ventricles, its use has largely been restricted to a regional topographical analysis of surface specializations. Such investigations, particularly of the third cerebral ventricle, have demonstrated regional differences in the surface of the ventricle walls consistent with the pattern of tanyocyte distribution demonstrated in light microscopic (LM) and transmission electron microscopic (TEM) investigations of equivalent ventricular areas, and with their functional specialization (re: sections 2, 4, 6). Using the SEM only, it has not been possible to distinguish tanyocytes from ordinary ependymal cells from a topographical analysis of their surface. We have attempted to demonstrate, therefore, the three-dimensional structure of the tanyocyte and its hypothalamic portal vascular termination with the SEM and to correlate these observations with the appearance of their surface. Such a study has not to our knowledge been undertaken previously.

Our observations were restricted to the non-ciliated region of the rabbit third ventricle at the level of the IR. The intricate structural relationship established between an infundibular ependymal cell process and a blood vessel of the hypothalamic portal system as viewed with the SEM is shown in Fig. 5. The cell body is rounded in shape (9-12 μm diam.) and its apex protrudes into the ventricular lumen. The luminal surface is non-ciliated with numerous irregular blebs or microvilli-like evaginations of the plasmalemma in accord with previous TEM and SEM descriptions (re: section 2 and 4). Occasionally more elongate flask-shaped tanyocyte somata were also discernible. A single long (0.1 mm) thick process extends from the base of the tanyocyte soma into the subjacent neuropil. This transition from cell body to tail process was often clearly definable, although this region was also often obscured by numerous interwoven processes whose origin could not be determined. The tail process is smooth and uniform throughout its extent, tapering (3.0-1.5 μm diam.) as it approaches the blood vessel. Although it appears unbranched throughout its course, the process divides at its termination into several slender branches (0.1-0.4 μm diam.) which entwine about the vessel and terminate as small foot processes. This investigation, in examining the morphology of the tanyocyte in three-dimensional perspective, demonstrates its close conformity to LM and TEM descriptions. It also supports the inference of earlier SEM investigations that the modified ependymal cells distinguished in this region of the ventricle are specialized ependymal tanyocytes.


Scanning electron microscopy has been increasingly employed in recent years to examine the morphological features of the ependymal lining the third cerebral ventricle in several mammalian species. Knigge and Scott (1970), initially reported ependymal specializations within the area of the rat IR consistent with available ultrastructural evidence of intense transfer activity. The scope of subsequent SEM investigations has been extended to include topographical analyses of the lateral cerebral ventricles and choroid plexus in the cat, Clementi and Marini (1972), Noack, Dumitrescu and Schweichel (1972), sheep Kozlowski, Scott and Murphy, (1972), and dog, Allen and Low (1973); the fourth cerebral ventricle and circumventricular organs in the rat, Torack and Finke (1971), rabbit, cat and squirrel monkey, Weindl and Joynt (1972a, b), as well as developmental abnormalities in rat ependyma and choroid plexus Chamberlain (1972). The unique morphological features of the ependymal surface of the rabbit, rat, mouse and human third ventricle have also been described Bruni, Montemurro, Clat­tenburg and Singh (1972). In all species, regional ependymal specializations suggestive of a secretory or absorptive capacity, were consistently observed within circumscribed areas of the ventricle.

A diagrammatic representation of the excised diencephalic block showing the entire rabbit third ventricle
in mid-sagittal section precisely as viewed with the SEM is provided in Fig. 6. The third ventricle can be conveniently divided into three regions on the basis of observed variation in the structural features of the ependymal surface. These divisions and the relative position of the underlying hypothalamic nuclei are illustrated in coronal section in the same figure.

In the rabbit, the upper two-thirds of the ventricular wall is lined by ependymal cells with a profusion of cilia that extend into the lumen of the ventricle; non-ciliated ependymal surface is infrequently observed (Fig. 7). This type of surface extends from the lamina terminalis and anterior commissure to the cerebral aqueduct caudally. Cilia project from the surface of each ependymal cell in clusters which often appear oriented in parallel longitudinal rows. In contrast, a transition in the morphology of the luminal ependymal surface occurs at about the level of the underlying ventromedial nucleus (Fig. 8). Cilia occur less frequently, and non-ciliated ependymal cells predominate. The latter are generally rounded in shape and their apices protrude into the ventricular lumen, forming a smoothly contoured surface. Similar surface specializations (ciliary crowns) have recently been demonstrated in the cilia of mouse oviduct with both the scanning and transmission electron microscope (Dirkson and Satir, 1972).

In the sheep, Kozlowski, Scott and Dudley (1973), monkey, Coates (1973a, b), and the mink, Scott, Kozlowski and Dudley (1973) there is also a terminal dilatation of the apex of each cilium, the diameter of which exceeds that of the ciliary shaft. The ciliary surface in the rabbit, however, is irregular and almost segmental in appearance, unlike that of either the mink, Scott et al. (1973), or the cat, Clementi and Martini (1972), which possess smooth profiles without particular substructure. Such morphology may reflect a means of extending the surface area for purposes of ciliary pinocytosis (Brightman, 1965). Occasionally, periciliary moats as described by Kohno and Usui (1966), could also be seen surrounding the base of each cilium at the cell surface. For a detailed discussion of the structure and function of cilia, the reader is referred to the following reports: Brightman and Palay (1963), Worthington and Cathcart (1963), Cathcart and Worthington (1964), Kohno and Usui (1966), Dalen, Schlaper and Mamoon (1971).

The lower one-third of the ventricular wall, represented by an area along the floor and lateral walls of the IR (Fig. 9), is devoid of cilia except for the occasional individual cilium. The most consistent structural feature is the undulating non-ciliated ependymal surface and the presence of numerous irregular sur-
Figure 9. Along the floor and lateral walls of the rabbit IR, only occasional single cilia are seen. The characteristic feature of this region is the large undulating non-ciliated ependymal surface with its numerous irregular surface blebs or knob-like protrusions. (E1, E2), ependymal cells.

X 5,200

face blebs or knob-like protrusions. This distinct regional variation in the morphology of the ependymal surface is consistent with both SEM Scott, Dudley, Gibbs and Brown (1972), Scott, Pau1 and Dudley (1972), and ultrastructural observations Tennyson and Pappas (1962), Brightman and Palay (1963), Klinker-fuss (1964), Rinne (1966), Anand Kumar (1968), Malinsky (1968), Knowles and Anand Kumar (1969), in a number of species. It is evident that this regional specialization of ependymal structure may be suggestive of a difference in functional capacity. Although as yet largely unconfirmed, the contention is that the ependyma, particularly in the region of the IR, exhibit the classical morphological features of an actively absorptive, secretory and/or transporting epithelium (re: sections 2, 6).

i) Intraluminal Neuronal Processes

The presence of biologically active compounds has been demonstrated in the cerebrospinal fluid Lurie and Weiss (1967), Margolis and Altszular (1967), Anand Kumar and Thomas (1968), Bagshawe, Hillary, Orr and Rushworth (1968), Heller, Hasan and Saifi (1968), Vorherr, Bradbury, Hoghoughi and Kleeman (1968), Linfoot, Garcia, Wei, Fink, Sarin, Born and Lawrence (1970), Siersbaek-Nielsen and Molholm Hansen (1970). In addition to the supposition that ependyma may discharge similar substances into the CSF, intraventricular neuronal processes have also been considered a potential mode of entry for active principles and have recently received considerable attention. Both light microscopic and transmission electron microscopic investigations have demonstrated the presence of CSF contacting neurons within the ventricular lumen of numerous vertebrates. Bulb-shaped CSF contacting nerve terminals projecting between ependymal cells have been described in the third ventricle of fish, Oztan (1967), Vigh-Teichmann, Vigh and Koritsanszky (1970a). The occurrence of typical dense-core vesicles in these cell processes and their topographical arrangement indicate that they arise from neurosecretory cells and probably secrete into the ventricle. Similar neurons with ventricular processes projecting from the ependymal lining into the third ventricle have also been demonstrated in amphibians, Diericks (1962), Smoller (1965), Peute (1969), and reptiles Ito (1965), Vigh-Teichmann et al (1970b, c). Analogous nerve terminals are also present in the central canal of the amphibian and avian spinal cord Vigh, Vigh-Teichmann and Aros (1970), Vigh, Vigh-Teichmann, Koritsanszky and Aros (1971). Isolated profiles arising from nerve cells lying in the subependymal tissue are also encountered both at the ependymal surface and closely interdigitated with ependymal cells in the rat, Brightman and Palay (1963), Mitro (1969), Fox, DeSalva, Zeit and Fisher (1948), ascribed a receptor function to similar fiber endings in the monkey third ventricle. Matsui and Kobayashi (1968), however, have suggested that unmyelinated axonal endings which protrude into the third ventricle are only found infrequently in the rat. On the other hand, intraventricularly-lying myelinated neuronal endings have been found in the mouse IR Wittkowskii (1969). In the third and fourth ventricles of the rabbit and cat brain, bulb-like processes of non-myelinated nerve fibers have been described Leonhardt and Lindner (1967), Leonhardt (1968), Leonhardt and Prien (1968), Leonhardt and Backhus-Roth (1969). The processes contain both the small clear, synaptic-type vesicles and larger dense-core vesicles, as well as quite distinctive large mitochondria. Between the plasmalemma of the bulb and the apical plasmalemma of the ependyma, desmosome-like junctions and synapse-like contacts are found. Not all cell processes seen at the luminal surface therefore belong to ependymal cells. Distinct bleb-like protrusions or irregularities of the free surface seen consistently along the lower wall of the third ventricle with the scanning electron microscope, may be homologous with the neuronal projections between ependymal cells from underlying nerve cells as documented by LM and TEM. Alternatively, many or all of the surface specializations may in fact be cytoplasmic protrusions of the ependymal plasmalemma itself, similar to those described in the rat and sparrow, Matsui and Kobayashi (1968), Usui (1968).

The presence of a variety of supraependymal structures have also been reported in the mammalian cerebral ventricles Schwantitz (1969). In some species, glial cells lie freely in the ventricle Leonhardt and Lindner (1967), while in others, microglial-like "spider" cells lie on the ependymal surface Bleier (1971). Nerve fibers situated supraependymally have also been found Fox et al (1948), Westergaard (1972). Recently the presence of curious supraependymal cells with branching processes within the ventricles of a number of species have been confirmed by several authors using the SEM. It is speculated that they may represent intraluminal neuronal processes, although at present the precise nature and function of these conceivable neuronal elements is obscure. Clementi and Marini (1972), using SEM techniques, described numerous small round formations on

J. E. Bruni

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the floor of the cat third ventricle morphologically similar to the club-like liquor contacting nerve endings demonstrated in the TEM investigations of Vigh-Teichmann et al (1970b). Distinct projections of underlying neural tissue were not observed, however, in SEM investigations of the ependyma in the lateral ventricles of sheep, Kozlowski, Scott and Murphy (1972). Cellular elements with branching processes that could be interpreted as nerve cells were also found in the cat third ventricle Clementi and Marini (1972), as well as on the ependymal surface of the squirrel monkey organon vasculosum and in the basal hypothalamus of the rabbit, Weindl and Joint (1972a, b). Multi-processed conceivably neural supraependymal cells have also been consistently observed along the floor and at sites in the IR of the monkey, Coates (1972), (1973a, b), mink, Scott et al (1973), and human, Scott, Paull and Dudley (1972), third ventricle. Previous SEM investigations conducted in this laboratory have failed to document the presence of supraependymal neurons within the third ventricle of any species examined Bruni et al (1972), (1973). Recently however, we have observed supraependymal structures with many long branching processes extending over the underlying ependymal surface in the rabbit third ventricle. Although these elements may be conceivably construed as neuronal, their occurrence was restricted to the rabbit where they were seen only infrequently. The extent of the intraluminal structures is shown in Fig. 10a. They were situated along the non-ciliated antero-lateral wall of the rabbit IR. Oval or spherical shaped cell bodies with a relatively smooth texture were particularly conspicuous (Fig. 10d). Generally, a single thick branching process extends from the cell body over the ependymal surface. Bifurcating processes, many of which seemed to extend into the underlying ependymal surface, could often be traced for their entire length (Fig. 10b). Frequently, processes were interwoven at their termination in a tangled synapse-like contact with a similar process originating from another supraependymal cell (Fig. 10c). Although it is not possible to establish the neuronal nature of these structures with the SEM, comparative TEM investigations support this supposition Noack et al (1972), Westergaard (1972). Nevertheless, in view of the inconsistency with which we have observed such structures, and their frequently observed continuity with the cut surface of the tissue, the possibility of preparational artifact cannot be precluded. Therefore, cautious interpretation of such SEM observations is warranted. At present, direct evidence for functional roles, classification, precise origin or detailed course of these supraependymal cells is lacking. It has been assumed, however, that such cells may be capable of regulating the functioning of ependymal cells, that they may be involved in...
5. Application of Silver Nitrate Staining to Scanning Electron Microscopy of Ependyma

A corollary of our earlier SEM investigations was to establish a means of identifying and delineating individual ependymal cells, thereby recognizing cell patterns and differentiating them from other structures. The present study therefore investigated the extent to which the classical silver technique could be successfully adapted to the study of ventricular ependyma. Microscopic studies on ependymal cell boundaries following treatment with silver nitrate have not been undertaken previously.

Silver nitrate staining itself, however, is an old histological technique used to demonstrate endothelial and epithelial cell boundaries 'en face'. Recent studies have endeavoured to establish the significance of the silver lines Florey, Poole and Meek (1959), and the mechanisms involved in silver staining Gottlob and Hoff (1968). The suitability of silver nitrate staining for demonstrating aortic endothelial cell borders with the SEM was shown by Garbarsch and Christensen (1970). More recently Geissenger (1972), also reported the use of the SEM in investigations of arterial intima following impregnation with silver nitrate.

Details of the procedure used to prepare specimens for both SEM and comparative light and transmission electron microscopy have been reported Bruni et al (1973). Wholemount preparations of the rabbit third ventricle, viewed from the surface with the light microscope show a clear and uniform network of intercellular silver lines mark the borders of irregular or polygonal ependymal cells (E1, E2) that vary in both size and shape. Individual ependymal cells exhibit a differential affinity for the stain (arrowheads). Note the prominent Golgi complex (G) which occupies a supranuclear position within each cell. Lines stained with silver nitrate, nuclei stained with Harris hematoxylin. X 2,050

As viewed with the SEM, the ependymal cells showed exactly the same distinct outlines (Fig. 12) which had been observed previously 'en face' with the light microscope. The most notable feature is the appearance of the silver lines, which are much lighter and stand out from the rest of the tissue. The particles of silver gave rise to a higher emission of secondary electrons in material examined with the SEM and, therefore, it is possible to detect silver lines relative to the surrounding tissue by virtue of the fact they appear brighter Daniel (1969), Geissinger (1972). The non-ciliated ependymal cells are polygonal, their apices protrude into the ventricular lumen, and the intercellular boundaries are delineated by elevated silver deposits (arrows) which are lighter and stand out from the rest of the tissue. X 6,300
tricular wall has long been recognized, their possible neuroendocrine involvement, however, has been the focus of interest only in recent years. Since Lofgren (1959) (1960), initially suggested that ependymal cells and CSF of the third ventricle may be involved in the regulation of adenohypophysial activity, much evidence of functional involvement has subsequently been adduced. Secretion by certain specialized ependyma into the CSF of the third ventricle has been described by many investigators Lévéque and Hofkin (1961), Vigh, Aros, Wenger, Kortsanszky and Cegledi (1963), Vigh (1964), Lévéque et al (1966), Knowles (1967), Matsui and Kobayashi (1968), Wittkowski (1969), Kobayashi Matsui and Ishii (1970). On the other hand, the concept of tanyocytes absorbing substances from the CSF and/or sub­ sersing a transport capacity has also been postulated Anand Kumar and Knowles (1967), Knowles and Anand Kumar (1969), Knigge and Scott (1970), Kobayashi et al (1970), Kobayashi (1972), Kobayashi, Wada and Uemura (1972). Apart from these theories, correlations between pituitary activity and ependymal cell morphology have also been documented. Lévéque and Hofkin (1962) observed that gonadectomy, adenreleactomy, hypophysectomy and cortisone treatment had no effect on the content of PAS-positive material in the ependymal cells of the rat IR. Cold-stress and propylthioracil-treatment however, increased and decreased their content respectively. In the Rhesus monkey, changes in ependymal tanyocyte morphology have been associated with age, sex, reproduction and estrogen administration Anand Kumar and Knowles (1967), Anand Kumar (1968), Knowles and Anand Kumar (1969). Similarly, seasonal changes in ependymal morphology associated with sexual activity have also been demonstrated in the skunk, Hagedoorn (1965). It has been suggested that ependymal cells might be involved in the production and/or transport of pituitary releasing factors Knigge and Scott (1970), Scott and Knigge (1970), Anand Kumar (1972), Knowles (1972), Lévéque (1972). The demonstration of neurohormones in the ventricular CSF Heller et al (1968), Vorherr et al (1968), Dencken and Haggendal (1969), Heller (1969), Linfoot et al (1970), Pavel and Coculescu (1972), and the transfer of active principles from the ventricle to the portal blood and pituitary Kendall, Grimm and Shimshak (1969), Knigge and Silver­ man (1972), Ondo, Mical and Porter (1972), Schecter and Weiner (1972), Ondo, Eskin, Mical and Porter (1973), indicates that ependymal mediation cannot be discounted. On the other hand, the questionable physiological significance of such a system Weiner, Blake and Sawyer (1971), Weiner, Terkel, Blake, Schally and Sawyer (1972), Gordon, Bolinger and Reichlin (1972), and the identification of hypothalamic sites of origin of releasing factors, other than ependymal Clattenburg, Singh and Montemurro (1971), (1972), Motta, Piva, Timo, Manis and Martini (1971), Timo (1971), casts some doubt on this supposition.

i) Effects of Ovariectomy on Ependyma

Kobayashi and Matsui (1969), and Kobayashi et al (1970), reported that 3 weeks after ovariectomy, most ependymal and hypendymal cells of the rat ME were enlarged and became cylindrical; their nuclei were also enlarged and cytoplasmic organelles were prominently increased in number. However, cytoplasmic protrusions and microvilli on the apical surface of the ependymal cells were poorly developed. Comparable results have been observed by Oksche, Zimmermann and Oehmke (1972) in a morphometric analysis of ependymal activity in the mouse arcuate nucleus and ME following ovariectomy. In contrast, Knowles and Anand Kumar (1969) reported striking regressive changes in the ventricular border of tanyocyte ependyma and in their contents 2 months following ovariectomy in the Rhesus monkey. Subsequent estrogen administration, noticeably increased the number of cytoplasmic protrusions and bulbous projections of the juxtaventricular surface.

**Figure 13.** Transmission electron micrograph of ventricular ependyma in the rabbit after staining with silver nitrate. Silver deposits along the entire lateral interspace between adjacent ependymal cells (Ei, E2) and randomly distributed silver particles can also be seen on the juxtaluminal surface (arrows). (V), third ventricle; (Nu), ependymal cell nucleus.

X 14,400
Figure 14. Scanning electron micrograph of the lateral wall of the rabbit IR three weeks after ovariectomy. The ependymal surface is irregular; individual ependymal cells are distinct, and appear enlarged and cylindrical. X 780

Inset, increased numbers of bleb-like protrusions and finger-like microvilli of various shapes are observed on the surface of each ependymal cell. X 3,300

In a series of experiments conducted in this laboratory, the morphology of the ependymal surface along the ventrolateral wall and floor of the rabbit third ventricle was examined with the SEM following ovariectomy. In normal females, the non-ciliated ependymal cells of this region are rounded in shape, and their juxtaventricular surface protrudes into the lumen forming a smoothly contoured surface (Fig. 8). Their apical surface is interrupted by small irregular knob- or bleb-like protrusions of the plasmalemma (Fig. 9). From 3 days to 34 weeks after ovariectomy however, this surface became more irregular; individual ependymal cells appear enlarged and cylindrical (Fig. 14). These morphological findings are consistent with the ultrastructural descriptions of Kobayashi and Matsui (1969), Kobayashi et al (1970), Oksche et al (1972). Individual ependymal cells are distinct and, in contrast to previous reports, increased numbers of bleb-like protrusions and finger-like microvilli with various shapes are observed on the ependymal surface (Fig. 14, inset). Although the functional implications of these morphological findings are uncertain, they have been interpreted to indicate an enhancement of ependymal absorption, secretion, and transport to the primary portal capillaries.

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