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The Surface Ultrastructure of the Habenular Complex of the Rat

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Summary. The surface features of the ependymal lining of the habenular complex in rats, aged between three weeks and nine months, were studied by means of scanning and transmission electron microscopy.

The ependyma of the medial habenular nucleus is heavily ciliated, the cilia obscuring underlying substructure in SEM – preparations. On the habenular commissure most cilia are arranged in tufts. Cilia are provided with segmental indentations and occasional apical thickenings. Vesicular protrusions of the ependymal cytoplasm into the ventricular lumen and the frequent occurrence of homogeneous supraependymal globules were interpreted as signs of ependymosecretory activity of nucl. hab. med. Supraependymal cells are most numerous on the anterior and superior surface of the habenular commissure. Cells presenting features identical to Kolmer (epiplexus) cells were identified on the ventricular surface of nucl. hab. med. in one specimen showing degenerative changes of undetermined aetiology in the habenular nuclei. It is therefore suggested that such cells need not necessarily be restricted to the choroid plexus.

Supraependymal unmyelinated axons are particularly numerous on both nucl. hab. med. and commiss. hab. They make desmosome contacts (maculae adherentes) with the ependymal plasmalemma. Contacts presenting all features of typical synapses were not encountered. The vesicle population of the axonal profiles mainly comprises 35–50 nm translucent round vesicles besides small numbers of 60–100 nm dense-cored vesicles and large pleiomorphic vesicles. Most probably the axons belong to the well-established dense population of serotonergic axons in the dorsal part of the third ventricle.

Key words: Habenula – Ependyma – Supraependymal cells – Supraependymal axons – Electron microscopy – Rat.

Introduction

It is well established that the habenular nuclei are forebrain-midbrain relay stations of major importance upon which influences from various limbic and – to a lesser extent – olfactory brain regions converge, mainly via the medullary stria. Efferent projections are preponderantly directed towards the interpeduncular nucleus and

several tegmental areas by way of the fasciculus retroflexus (habenulo–interpeduncular tract). With respect to the connections of the mammalian habenular nuclei numerous reports recently have appeared, based mainly upon degeneration- and neuro-physiological methods, to a lesser degree upon retrograde HRP transport, autoradiographic fiber tracing and histochemical methods.

Summing up, the most significant of these reports have yielded the following concept of habenular connections.

Afferent to nucl. hab. med: posterior septum (Cragg, 1961), superior cervical sympathetic ganglion (Björklund *et al.*, 1972; Moore, 1975), postcommissural septum, nucleus of diagonal band and mesencephalic raphe (Herkenham and Nauta, 1977).

Afferent to nucl. hab. lat.: septum and lateral preoptic area (Mok and Mogenson, 1972a, b), preoptic area, anterolateral dorsal thalamus and opposite nucl. hab. lat. (Cragg, 1961), lateral hypothalamus, hippocampus, amygdala and olfactory bulb (Mok and Mogenson, 1974), globus pallidus (Nauta, 1974), entopeduncular nucleus, lateral preoptico-hypothalamic region, nucleus of diagonal band, substantia innominata, ventral tegmental area, mesencephalic raphe and central gray substance (Herkenham and Nauta, 1977).

Afferent to nucl. hab. (subnuclei not specified): lamina intercalaris (sympathetic, Wiklund, 1974).

Nucl. hab. med. efferent to: interpeduncular nucleus (Kuhar *et al.*, 1975: cholinergic, Mroz *et al.*, 1976: substance P), interpeduncular nucleus, central superior and dorsal raphe nuclei, ventral tegmental nucleus, diverse thalamic nuclei, posterior medial septum and amygdala (Akagi and Powell, 1968).

Nucl. hab. lat. efferent to: dorsal and median raphe nuclei (Aghajanian and Wang, 1977), dorsal and median raphe nuclei and caudal central gray (Pasquier *et al.*, 1976), dorsal tegmental nucleus, pretectal area, superior and inferior colliculus, preoptic area and interpeduncular nucleus (Akagi and Powell, 1968).

Nucl. hab. efferent to (subnuclei not specified): interpeduncular nucleus (Kataoka *et al.*, 1973: cholinergic), interpeduncular nucleus, ventral tegmental nucleus, central gray (Way, 1975), interpeduncular nucleus, lateral preoptic and lateral hypothalamic area, dorsomedial and parafascicular nucleus (Yamadori, 1969), anterior thalamic nuclei, hypothalamus, preoptic area, amygdala, diagonal band of Broca, innominate substance of Reichert, (probably) septal nuclei (Mitchell, 1963), pineal ganglion (David and Herbert, 1973).

In a recent, detailed Golgi-study of the cat habenular nucleus Iwahori (1977) classified the neurons on a basis of perikaryal size and shape and dendritic morphology. Nuclear areas of the nucl. hab. lat. and the nucl. hab. med. appeared to receive fundamentally different groups of afferent fibers.

In view of the extensive literature mentioned above, it is striking that the ependymal surface of nucl. hab. med. and the habenular commissure apparently have not been subjected to detailed electron microscopical investigation, more particularly scanning electron microscopy. Ependyma and supraependymal surface specializations of various other areas bordering on the third ventricle have been intensively studied (see Discussion), the findings obtained frequently yielding important clues as to functional aspects of the underlying nuclear field (*e.g.* neuroendocrine regulation, ependymosecretion, regulation of ciliary activity). Therefore, a study of the surface morphology of the habenular complex was considered a worthwhile addendum to the reported studies concentrated upon connections and internal structure of this region.

Material and Methods

Sprague–Dawley rats of both sexes were fixed under sodium pentobarbital (Nembutal) anaesthesia by cardiac perfusion using a volume of approximately 300 ml of a solution of 2% glutaraldehyde in 0.1 M phosphate buffer at pH 7.38 (about 500 mOs) following perfusion with a buffered saline solution containing 2% PVP (polyvinyl–pyrolidon) to which 0.4% sodium nitrite had been added. After immersion fixation in fresh cold 2% glutaraldehyde fixative overnight, the brains to be processed for SEM were sagittally split to expose the third ventricular walls, and the habenular regions were resected. After rinsing in saline and distilled water the specimens were dehydrated in five steps with graded acetone and further desiccated in a critical-point apparatus (Polaron) using Freon 13. The dried and mounted tissues were subsequently exposed to osmium tetroxide vapour during three days. In a vacuum evaporator the blocks were coated with gold prior to examination and photography in a Jeol JSM-U_3 scanning electron microscope operating at 25 KV.

The SEM material comprised the following brains: nine months (9), three months (2), 46 days (4), six weeks (3), three weeks (4; 3 excluded because of artifacts).

In the TEM procedure perfusion and immersion fixation was performed as for SEM. Sections, 30 μm thick, of habenular regions were cut using a Vibratome-apparatus. Selected regions were postfixed in 2% osmium tetroxide phosphate buffered at pH 7.4 for 1 h., dehydrated and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate solution for 30 min and examined with a Philips 300 transmission electron microscope.

The TEM material comprised the following brains: nine months (4), four months (2), three months (2), 46 days (1), six weeks (7).

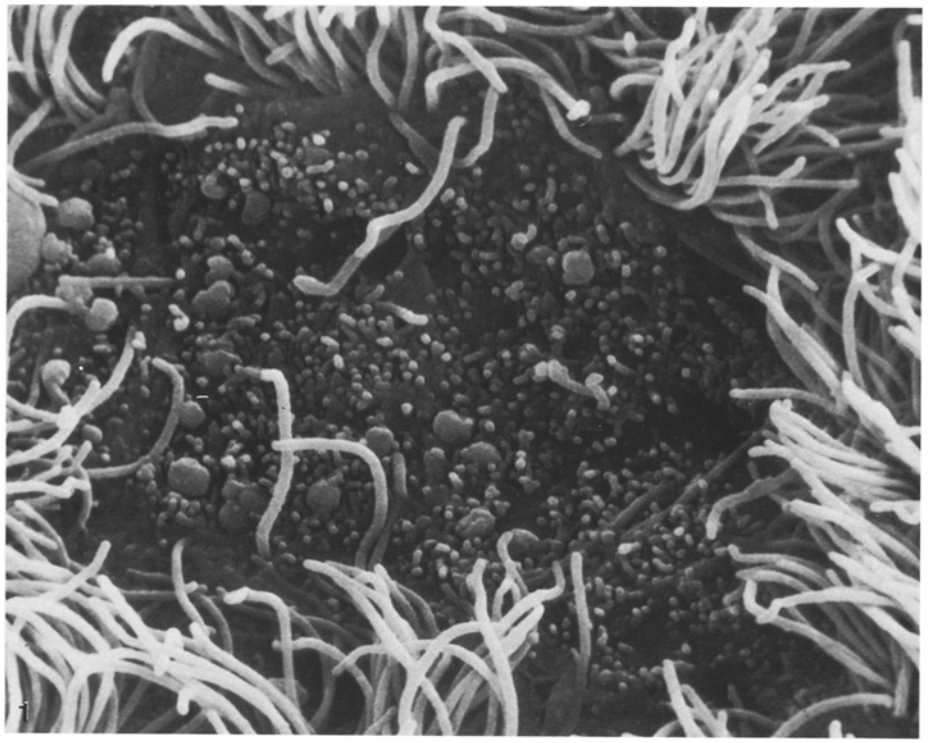
Results

Cilia – nucl. hab. med. [*SEM*]

In all specimens the ventricular surface of nucl. hab. med. was almost completely covered with a dense population of cilia. They largely obscure underlying structures including microvilli. Small areas exhibiting a lesser ciliar density are mainly located near the stria medullaris (Fig. 1). The shaft diameter, as measured from 150 cilia halfway along the ciliar length, averaged 0.23 μm in both three and nine months stages. A minority of cilia is provided with apical thickenings, mostly spherical but occasionally discoidal in shape (Fig. 2). The knobs show marked individual variation as to form and number. Most cilia demonstrate segmental indentations (Fig. 3). Knobs and indentations were observed in all specimens. The overall ciliar arrangement appears to be similar to that on the adjacent dorsomedial thalamic nucleus, the ventricular surface of which was exposed consequent to the medio-sagittal sectioning of the brains. In three specimens of the nine months age group a repetitive wave-like arrangement suggestive of motility in metachronal waves at the time of fixation was a prominent feature (Fig. 4). In all other specimens, however, no pronounced order in the ciliar array could be detected, possibly due to distorting influences from the histological procedure.

Cilia – commiss. hab. [*SEM*]

In contrast to nucl. hab. med., the overall ciliar density is small. Most cilia, in all stages studied, were typically assembled in groups (Fig. 5). The average number of cilia in 50 inspected groups amounted to 30 in both the three and nine months stages. The shaft diameter as measured from 150 cilia halfway along the ciliar length averaged 0.35 μm in both the three and nine months stages and thus surpasses that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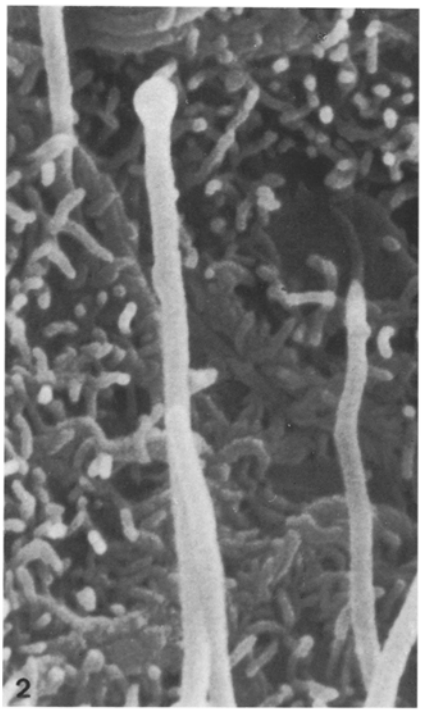
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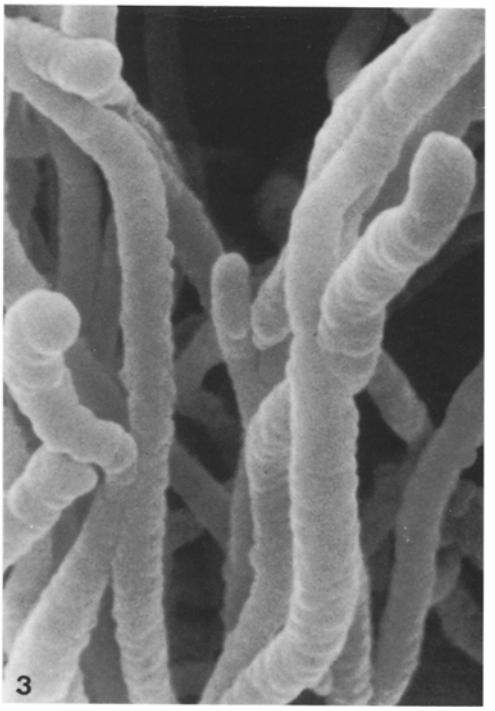
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of nucl. hab. med.-cilia. Apical enlargements and segmental indentations are similar to those of nucl. hab. med.-cilia. Between the ciliar tufts isolated cilia are irregularly distributed. In these regions the ependyma is seen to be provided with heavy aggregations of microvilli and supra-ependymal axons (SEA's) (see further on) (Fig. 6). The diverse surface regions of commiss. hab. all demonstrate the same overall pattern of ciliar population. The ciliar ultrastructure as displayed by TEM conforms to the well-known normal configuration (9 plus 2 array) of ependymal cilia and therefore will not be commented upon.

Secretory phenomena [*SEM, TEM*]

In SEM-preparations of nucl. hab. med. in sparsely ciliated areas spherical formations were observed between the microvilli. It could not be ascertained whether they were continuous with the ependymal cytoplasm. They could clearly be differentiated from the microvilli. Similar structures were not observed on the commiss. hab. The TEM equivalent of these globules appeared to consist mainly of supraependymal, circular or oval, isolated profiles filled with evenly dispersed and finely granular, moderately electron-dense material, occasionally demonstrating a flocculent appearance (Fig. 7). Besides, in these profiles pleiomorphic electron-lucent elements of varying calibre may be present. In a six weeks old specimen a circular profile was identified, measuring 880 nm in diameter, being constituted of a large number of electron-lucent vesicles (Fig. 8). The production of these globules by ependymal secretory activity is suggested by the frequent occurrence of bleblike protrusions of the ependymal plasma membrane into the ventricular lumen, the protrusion possessing an identical ultrastructure. Such large excrescences were often observed to be connected with the ependyma by a slender cytoplasmic bridge (Fig. 19), a feature suggestive of a pinching-off process.

Supraependymal Cells – nucl. hab. med. [*SEM*]

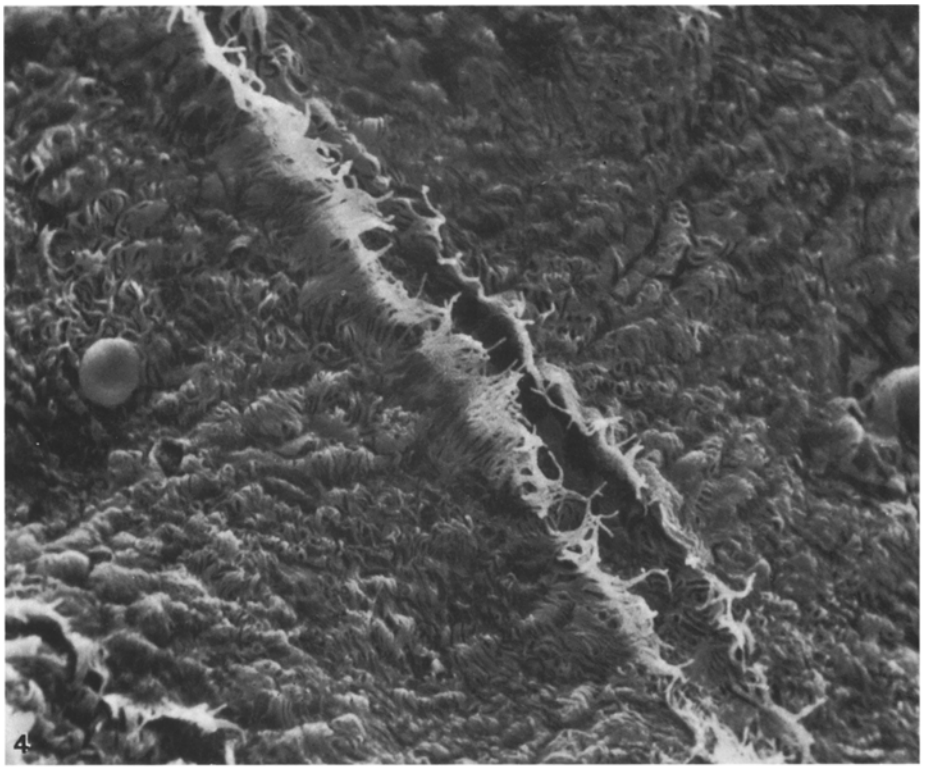
Supraependymal cells (SEC's) were identified in SEM-preparations of the nine months stage. Their actual numbers could not be established because their visualization is severely hampered by the profuse ciliar population. The smooth-surfaced perikaryon is usually spherical or ovoid in shape and emits a small number of processes. The long axis and the largest perpendicular axis of ovoid cell bodies averaged 6.5 and 5.7 μm respectively. The diameter of the processes surpasses considerably that of the supraependymal axons (discussed further on). The great ciliar density did not allow tracing of the processes up to their termination. Unfortunately, in TEM-sections profiles of these cells were not observed.

An interesting observation, to be discussed briefly, was made in TEM-sections from one, 46 days old, specimen only. Great numbers of polymorphic macrophages

Fig. 1. In small, nonciliated areas of the surface of the medial habenular nucleus, the ependyma is seen to be provided with microvilli and bulbous protrusions. Three months old rat, ×3,800

Fig. 2. Cilium provided with apical thickening. Medial habenular nucleus. Six weeks old rat, ×12,000

Fig. 3. Cilia on the medial habenular nucleus exhibiting segmental indentations. Three months old rat, ×20,000



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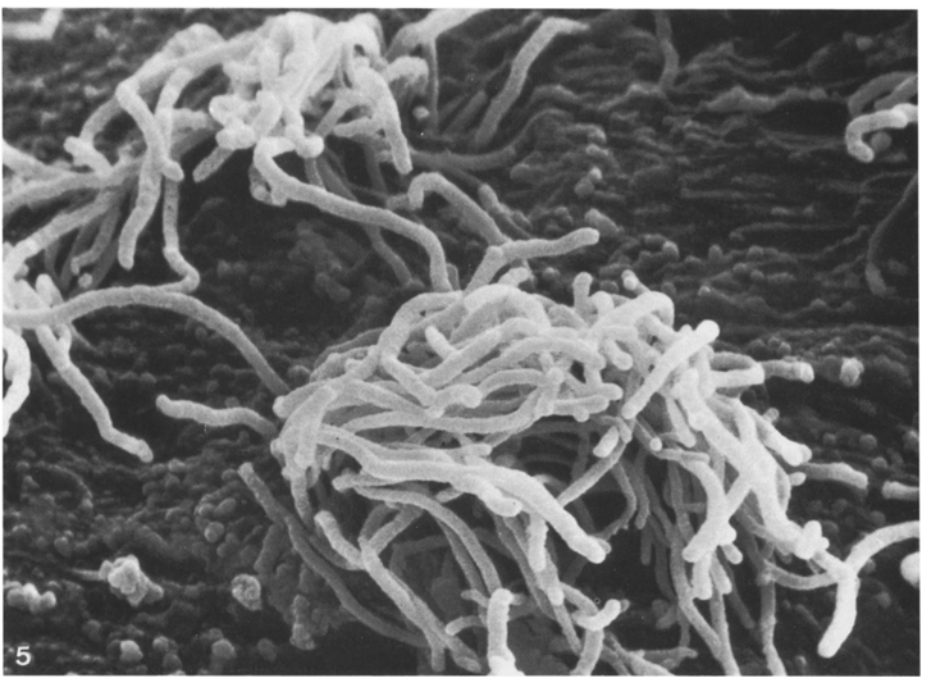

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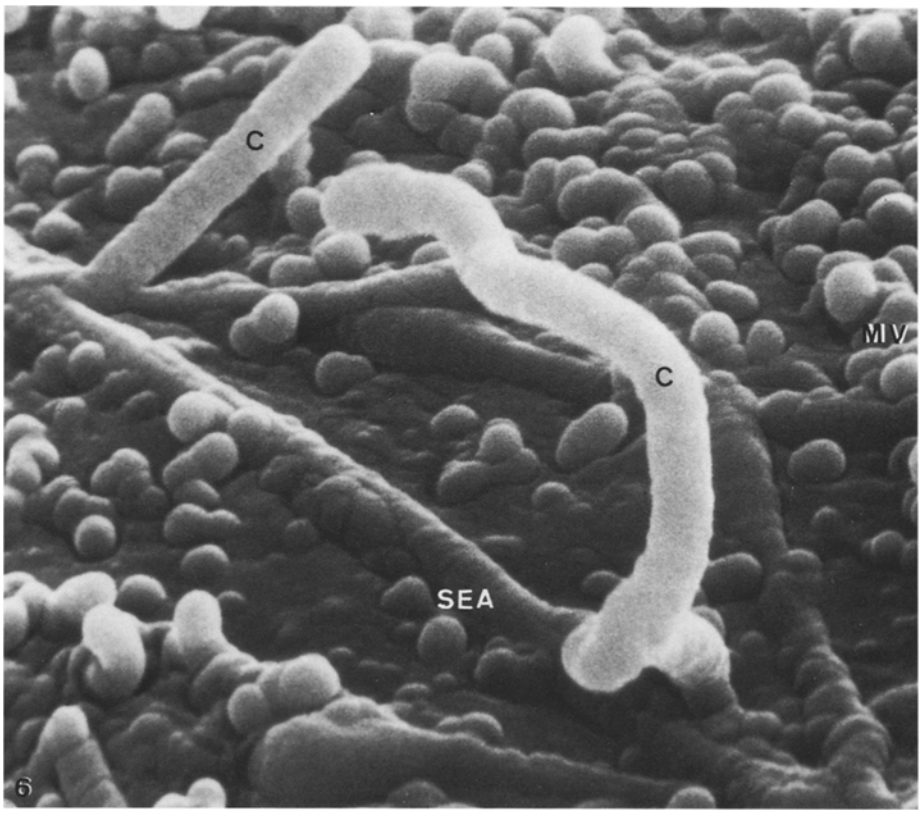
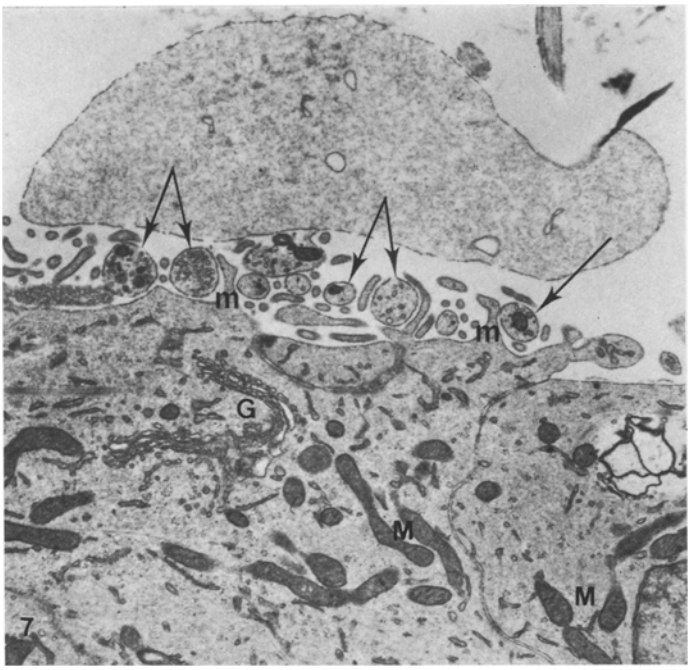


Fig. 6. Large areas of the superior surface of the habenular commissure are relatively devoid of cilia. Between isolated examples (*C*), microvilli (*MV*) and supraependymal axons (*SEA*) are conspicuous elements. Nine months old rat, ×16,000

were seen to be juxtaposed to the ventricular surface of nucl. hab. med. (Fig. 9). Most profiles have highly irregular contours (Fig. 10). Therefore, and because of their location on top of the ciliar population, these cells evidently are not equivalent to the smooth-surfaced cells found directly apposed to the ependymal cell membrane in SEM-specimens. In the usually marked eccentrically located nucleus, coarse chromatin substance is condensed beneath the nuclear membrane. Aside from the Golgi-apparatus and mitochondria, prominent features in the cytoplasm are: long cisternae of endoplasmic reticulum, membrane limited electronlucent circular profiles, vacuoles, microvesicles and lysosomal dense bodies (Figs. 10, 11). In a number of cells the plasma membrane is deeply indented by underlying habenular

Fig. 4. Wave-like arrangement of cilia on medial habenular nucleus, suggestive of co-ordinated motility at time of fixation. Nine months old rat, ×1,400

Fig. 5. Ciliar arrangement in tufts on anterior surface of habenular commissure. Between the tufts the ependymal plasmalemma, covered with microvilli, is visible. Nine months old rat, ×6,000



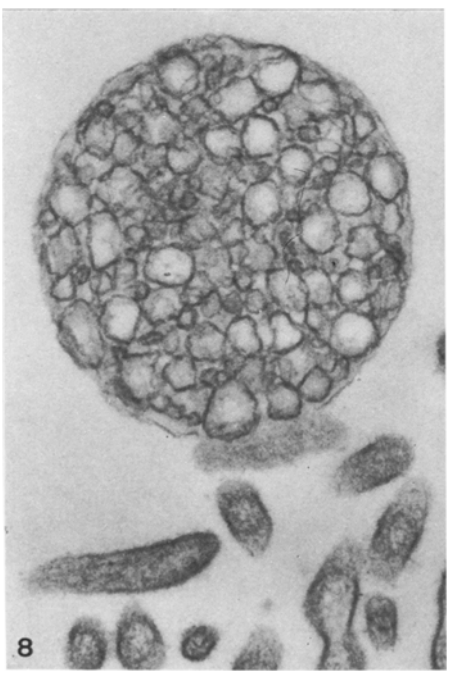
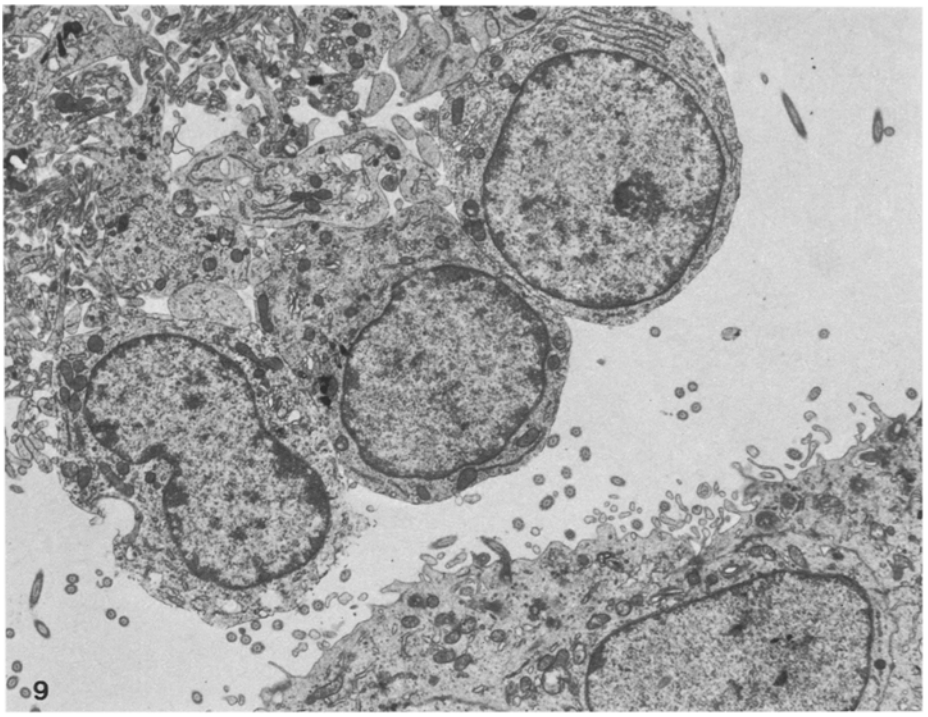


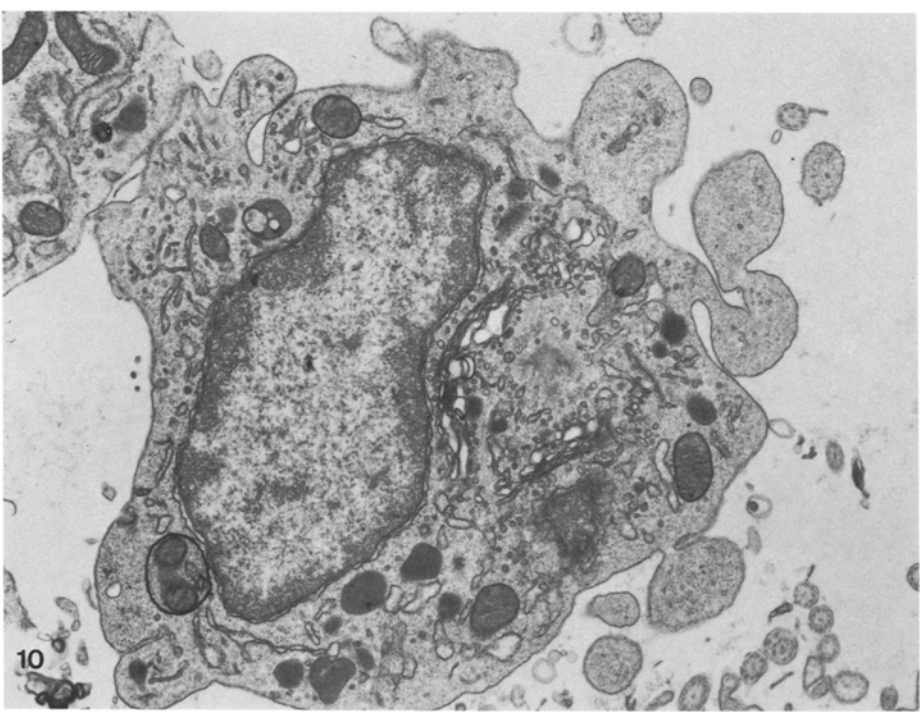
Fig. 7. The large, homogeneous and finely granular supraependymal mass may be a habenular secretion product. Many axons (arrows) are situated on the apical ependymal plasmalemma of the medial habenular nucleus. *G*, Golgi-apparatus; *M*, mitochondria; *m*, microvilli. Six weeks old rat, ×15,000

Fig. 8. Circular profile of multivesicular globule, interpreted as a secretion product, above medial habenular nucleus. Six weeks old rat, ×60,900

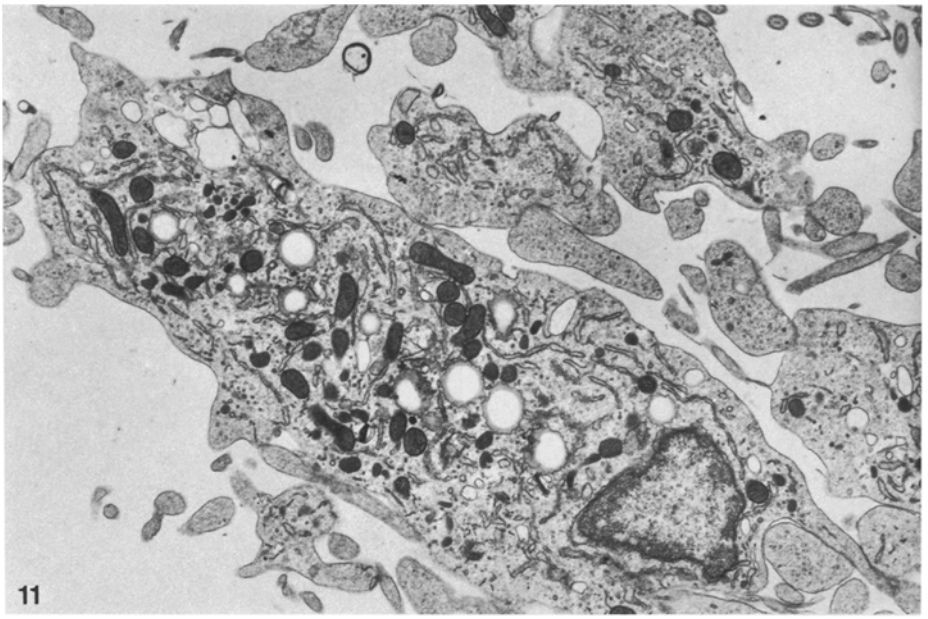
Fig. 9. Supraependymal macrophages, identified as Kolmer cells, juxtaposed to ventricular surface of medial habenular nucleus. 46 days old rat, ×7,000

Fig. 10. Supraependymal Kolmer cell of medial habenular nucleus. Note highly irregular contour of





cell membrane, aggregation of chromatin beneath nuclear envelope, and presence of lysosomal dense bodies, mitochondria, Golgi elements, vacuoles and microvesicles in the cytoplasm. 46 days old rat, ×15,000



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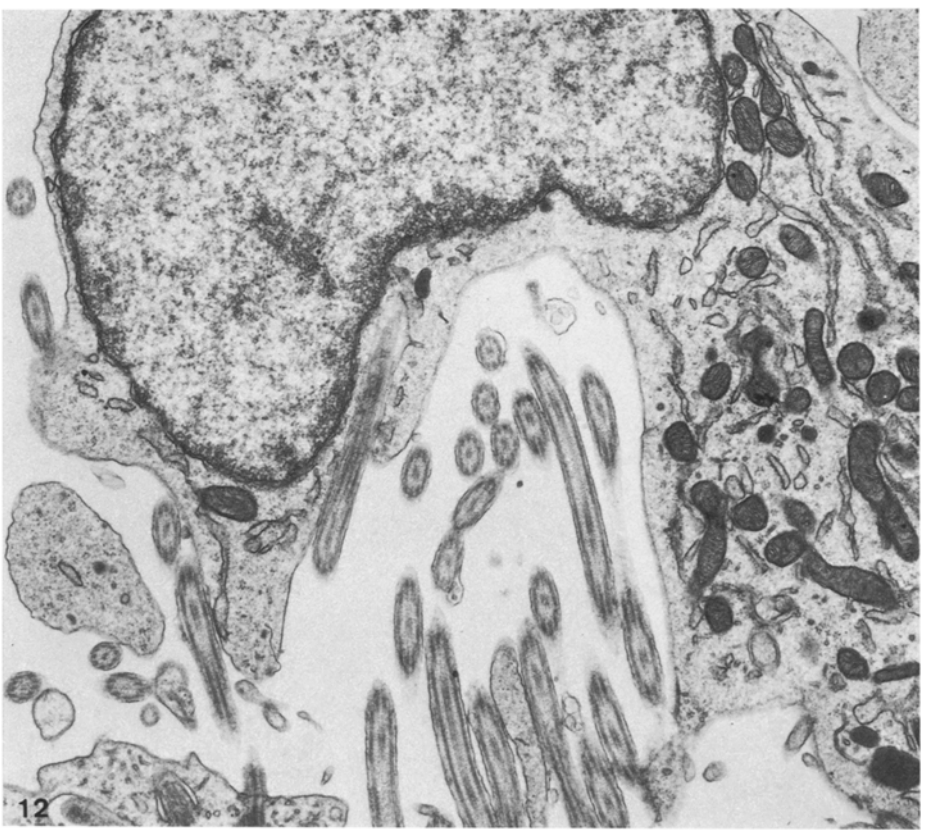

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cilia (Fig. 12). The structural details mentioned are typical for Kolmer (epiplexus) cells. In this particular brain both ependyma and neurons of the nucl. hab. showed clearcut degenerative changes of obscure aetiology.

Supraependymal Cells – commiss. hab. [*SEM*]

In virtually every specimen of all stages investigated a considerable number of cells located on the ependyma of the commissure, not being obscured by cilia, was readily identified (Figs. 13, 14). Most were encountered on the anterior and superior surfaces of the commissure, but they do not seem to be restricted to special areas. Within each age group the number of cells varies. Besides, a left-right asymmetry as to number of cells is frequently apparent. In nine months old rats the largest visible cell diameter and the largest perpendicular axis averaged 7.7 and 5.5 μm respectively. Most perikarya are ovoid shaped, some spherical, and have a smooth surface. Most cells lie isolated; a minority is arranged in groups. A maximum number of cells per group (15) was noted on the anterior commissural surface of a 46 days old rat (Fig. 15). The number of primary cell processes observed amounted maximally to six and averaged three. No prolongations were unequivocally observed to penetrate through or between ependymal cells. Most processes extend for considerable distances and emit collateral branches.

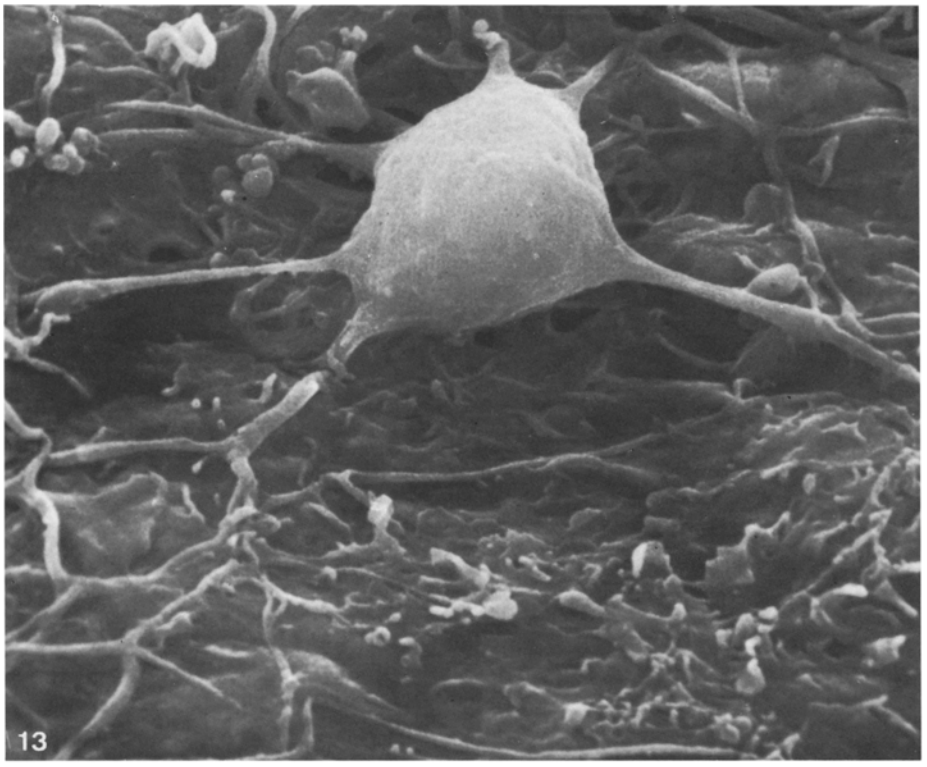
Supraependymal Fibers [*SEM, TEM*]

In all SEM-specimens from each age group, great numbers of fibers were observed on the ependyma of the whole habenular complex (Fig. 16). They are easy to differentiate from incidentally occurring creases of the ependymal plasmalemma. On nucl. hab. med. the fibers are largely hidden from view by the cilia, but in restricted, sparsely ciliated areas they are readily demonstrated. On commiss. hab. nearly all fibers are easily visualized related to the much lesser ciliar density. Branching points and overcrossings can occasionally not be discriminated. A minority of the fibers possess spindle shaped swellings.

A correlative TEM-study was especially performed in order to elucidate the nature and ependymal relations of the fibers observed in SEM. In all TEM-sections studied, round to oval profiles juxtaposed to or located at some distance from the ependymal plasmalemma were present in great numbers (Fig. 7). They were identified as axons on account of their regular round contour in cross sections, and because of the presence of prominent and evenly distributed 20–26 nm microtubules, mitochondria, synaptic vesicles and absence of ribosomes. A minority of the axons was sectioned tangentially, the majority more or less perpendicularly. Myelin sheaths were not observed. Many axons are assembled in groups. Sometimes axons are

Fig. 11. Supraependymal Kolmer cell and parts of adjacent ones of medial habenular nucleus. In the cytoplasm are especially prominent mitochondria, cisternae of agranular endoplasmic reticulum, vacuoles and membrane limited electron-lucent circular profiles. The nucleus is excentrically located. 46 days old rat, ×9,800

Fig. 12. Another specimen of supraependymal Kolmer cells of medial habenular nucleus. The plasma membrane is invaginated by a bush of cilia. The nucleus is excentrically located. 46 days old rat, ×17,000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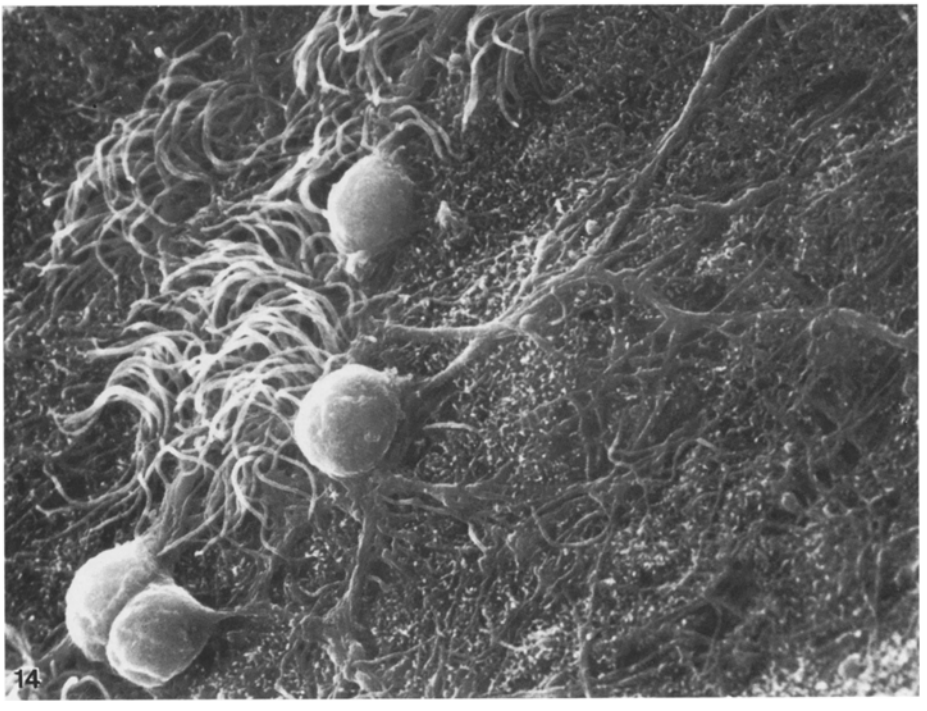
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arranged one on top of the other with regular intervals between them (Fig. 17). Axonal profiles with identical features were occasionally observed within the ependymal cytoplasm, but continuity between such intraependymal axons and SEA's could not be established.

The most commonly occurring synaptic vesicle profiles are translucent and roughly circular in shape, and measure between 35 and 50 nm (Fig. 18). Many axons contain also elongate translucent vesicles measuring approximately 20 nm in width and between 40 and 80 nm (mean 50 nm) in length. It has to be considered, however, that flattening of spherical synaptic vesicles can have occurred consequent to the aldehyde fixation and may be dependent upon osmolarity of the solution in which the fixative is dissolved and the solution used for washing the tissue prior to osmication, well-known artefactual effects. Of incidental occurrence are large, 80–170 nm oval and translucent profiles of agranular endoplasmic reticulum. Dense-cored vesicles are present in most axonal profiles in addition to the relatively small synaptic vesicles. They measure between 60 and 100 nm (mean 65 nm). Per circular axonal profile a maximum of five dense-cored vesicles was noted and an average number of about one. The number of mitochondria per circular axonal profile amounted to approximately one on average, and maximally four. The organelles of the nucl. hab. med.- and commiss. hab.-SEA's appear to be similar in relative abundance.

Most SEA's and the ependymal cell membrane are separated by an evenly wide cleft between 15 and 40 nm in width (mean 20 nm) (Fig. 19). A considerable percentage of profiles demonstrate features of terminals, such as increased numbers of synaptic vesicles and mitochondria. Many axons and terminals exhibit desmosomal contacts (maculae adherentes) with the ependymal plasmalemma (Figs. 20, 21). These contacts are characterized by symmetrically disposed densities along the surfaces of the apposed membranes and a filamentous gap substance of intermediate density in the intervening space. The cleft is approximately 20 nm in width. The thickening of the ependymal membrane sometimes encroaches upon a microvillus that is also apposed to the contacting axon. No synaptic vesicles are intimately related with these junctions. Genuine synaptic contacts presenting both accumulations of vesicles near to the contacting membranes and a 20 to 30 nm wide cleft and symmetrical or asymmetrical thickenings of apposed membranes, were not observed. Incidentally, accumulations of vesicles close to the side of ependymal contact were observed in boutons. In these boutons, however, a synaptic membrane thickening was not observed.

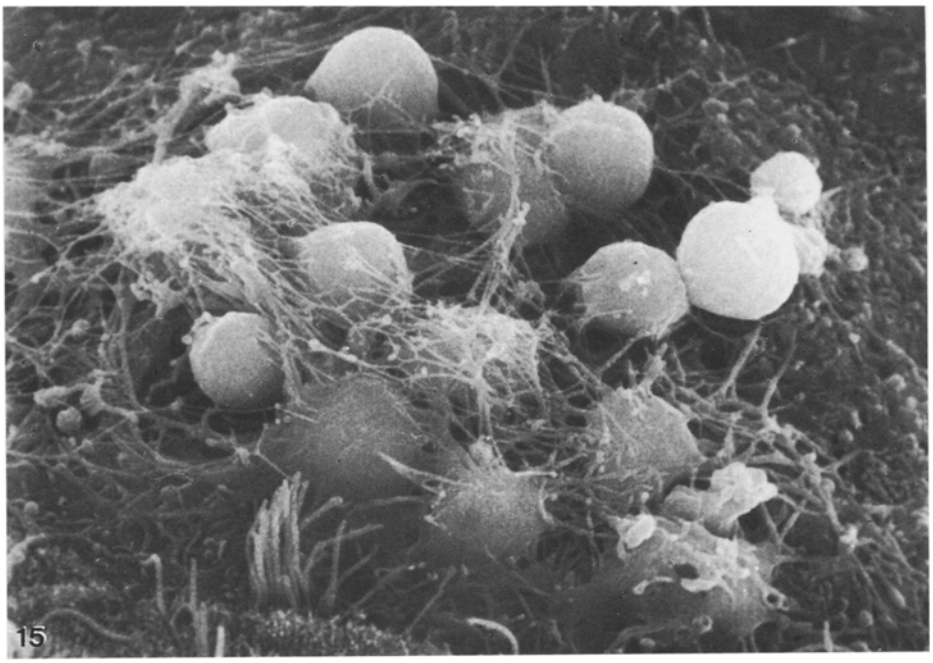
Discussion

Ciliar Population

The occurrence of a heavy ciliar population on nucl. hab. med. might have been inferred from reports indicating that the dorsal third of the third ventricular wall is

Fig. 13. Supraependymal cell of superior surface of habenular commissure. The processes can be traced for long distances. Six weeks old rat, ×6,130

Fig. 14. Supraependymal cells on superior surface of habenular commissure. Most or all of the branching fibers in the right part of the figure do not originate from such cells, but – as is evident from TEM-specimens – are axons of an as yet undetermined nature. Nine months old rat, ×2,530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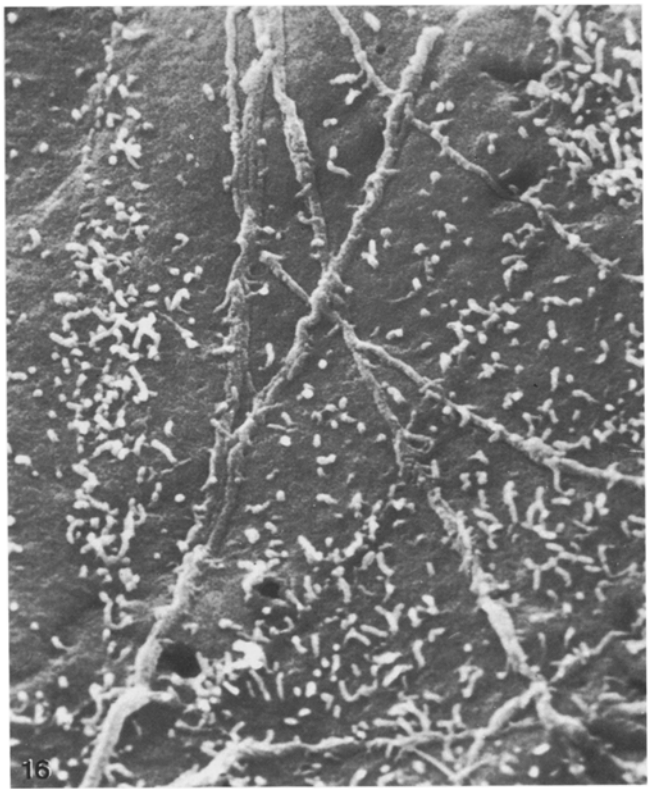
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characterized by heavily ciliated cells, the cilia obscuring underlying substructure (Bruni *et al.*, 1972: human, rabbit, rat, mouse; Kozlowski *et al.*, 1973: sheep; Scott *et al.*, 1972: human; Scott *et al.*, 1973: mink; Scott *et al.*, 1974: mammals in general). The commiss. hab.-ciliar arrangement, mainly in isolated groups, clearly deviates from this arrangement.

Terminal ciliar knobs in the habenular region seem to be identical to the bulbous enlargements observed in the dog lateral ventricle (Allen and Low, 1973), and the third ventricle of the rabbit (Bruni *et al.*, 1972), mink (Scott *et al.*, 1973, 1974) and monkey (Coates, 1977). Only very occasionally, terminal biconcave discs as observed by Kozlowski *et al.* (1973) in the sheep hypothalamus were encountered.

Secretory Phenomena

The observed cytoplasmic protrusions of the nucl. hab. med.-ependyma and the isolated supraependymal finely granular formations most probably are expressions of ependymosecretory activity.

Morphological evidence for ependymosecretion has been found in diverse regions of the vertebrate ventricular system. For instance, ependymal cells producing Gomori-positive secretory material have been identified in a special ependymal territory ("recessus organ") at the bottom of the third ventricle (Teichmann *et al.*, 1966; Vigh, 1964)and in the subcommissural organ (Vigh *et al.*, 1967).

In the floor of the infundibular recess of the mouse Wittkowski (1969) demonstrated a micro-apocrine secretion of tanycyte-ependyma. His description and illustrations of the pinching-off process of apical cytoplasmic protrusions and their subsequent expulsion into the liquor has many features in common with the findings in the habenular region. Multivesicular supraependymal secretory globules in the infundibular recess (Wittkowski's Fig. 8) and in the habenular area (Fig. 8 of present paper) demonstrate an identical structure.

Noteworthy is the observation of Kumar and Anand Kumar (1975) that the rhesus monkey habenular ependyma exhibits secretory activity in the form of cytoplasmic protrusions off-pinching into the cerebrospinal fluid. This was demonstrated in sexually immature and in menstruating animals. Homogeneous supraependymal cytoplasmic protrusions recently have also been observed by Kiss and Mitro (1976) in the mesencephalic ventricle of the hamster. In the lateral ventricle of Bradypus tridactylus, Ferraz de Carvalho and Costocurta (1976) also noted ependymal protrusions, some of which seemed to be liberated into the cerebrospinal fluid.

Supraependymal Cells

The existence of supraependymal cells (SEC's) on the habenular complex is no unique feature. Such cells have been demonstrated in various other ventricular regions. In the lateral ventricle they have been reported to occur by Allen and Low

Fig. 15. Cluster of spheroidal supraependymal cells of anterior surface of habenular commissure. They are partially enmeshed by fine threads of undetermined nature, 46 days old rat, ×3,060

Fig. 16. Supraependymal axons of superior surface of habenular commissure. Six weeks old rat, ×6,660

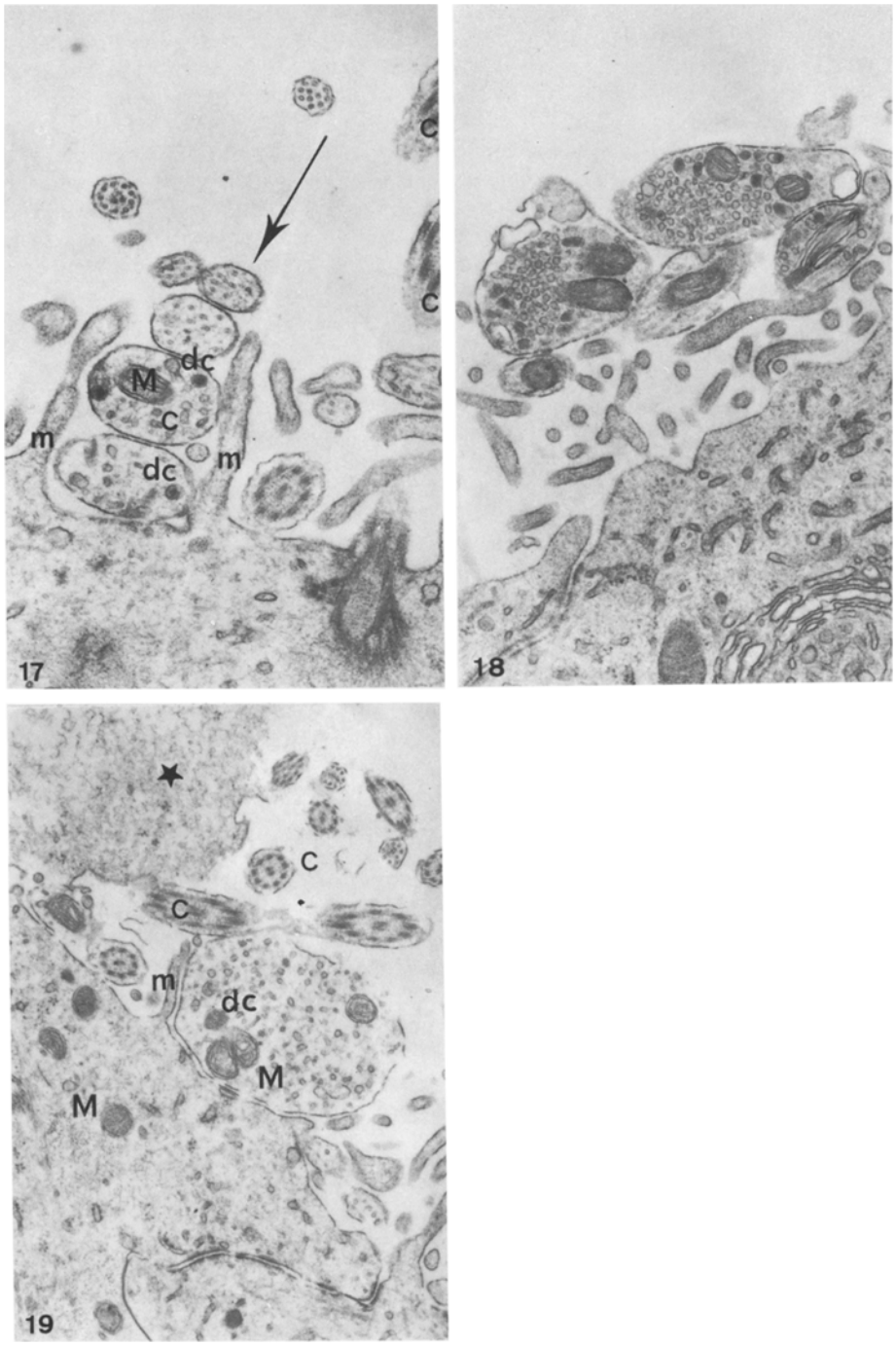


Fig. 17. Ependymal surface of medial habenular nucleus. Between two microvilli, four supraependymal axons are located on top of each other (arrow). *c*, clear synaptic vesicles; *C*, cilia; *dc*, dense-cored vesicles; *M*, mitochondrium; *m*, microvilli. Three months old rat, ×29,920

(1973) (dog) and would probably represent both neuronal and glial elements. Here they were also observed by Noack *et al.* (1972) (cat). In the fourth ventricle of the rabbit, SEC's would be represented mainly by glial cells, a few nerve cells participating in the organization (Leonhardt and Lindemann, 1973).

In diverse regions of the wall of the third ventricle other than the habenular surface, SEC's are known to occur. Coates (1973a, b) demonstrated in the preoptic and infundibular recesses of the monkey multiple branching and interweaving processes from SEC's. Some of these processes penetrate the ependyma, but the fine structure determined from TEM would not allow them to be classified as neuronal or glial. In the monkey and rabbit hypothalamus SEC's would resemble small neurons (Weindl and Joynt, 1972). Mestres and Breipohl (1976) interpreted SEC's in the rat hypophyseotropic area as mesenchymal cells that might have phagocytotic functions, being involved in renewal of ependyma. On the cat third ventricular floor, long and tortuous processes from SEC's were observed to form a sort of network and interpreted as neuronal by Clementi and Marini (1972). A comparable network of branches from SEC's is present on the organum vasculosum laminae terminalis (Weindl *et al.*, 1975) and in the infundibular recess (Martinez-Martinez, 1975). Stellate neuron-like cells located especially in the infundibular recess have been reported in the mink (Scott *et al.*, 1973, 1974).

Since, in the present study, no TEM-observations of habenular SEC's as identified in SEM, have been made, a classification of these cells as neuronal or glial seems inappropriate. Moreover, the material studied did not yield any clue as to the functions of these cells.

The ultrastructure of the supraependymal macrophages identified on the ventricular surface of nucl. hab. med. in one, 46 days old, animal correlates with the description of the ultrastructure of Kolmer cells of the cat choroid plexus by Carpenter *et al.* (1970) and with the illustration of a Kolmer cell of the rat choroid plexus by Peters *et al.* (1976).

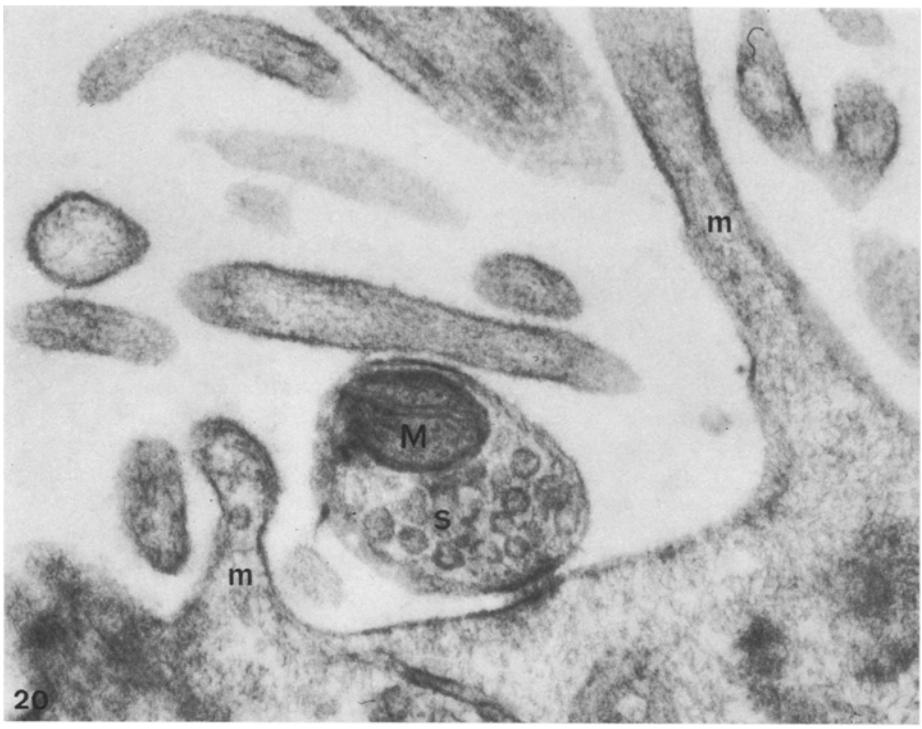
Apparently Kolmer cells need not be restricted to the choroid plexus, as has also been stated by Carpenter *et al.* (1970). Taking into account the degenerative changes of obscure aetiology in the habenula of the animal concerned, it seems likely that Kolmer cells have become detached from the choroidal epithelium to become involved in phagocytosis of habenular debris. Phagocytotic activity of Kolmer cells has experimentally been demonstrated by Ariëns Kappers (1953), who proposed designating these epi-epithelial plexus-macrophages as epiplexus cells.

Supraependymal Fibers

Several TEM- and SEM-studies have demonstrated supraependymal fibers, designated as neuronal and/or glial in the third ventricle. Concentrations are

Fig. 18. Supraependymal axons of medial habenular nucleus. Two profiles show high concentrations of clear 35–50 nm spherical vesicles and small numbers of 60–100 nm dense-cored vesicles. Some profiles of smooth endoplasmic reticulum are also present. Six weeks old rat, ×35,100

Fig. 19. Ependymal surface of medial habenular nucleus. A large axonal profile filled with pleiomorphic vesicles is separated from the ependymal plasmalemma and a microvillus by an evenly wide cleft. The finely granular, flocculent mass (*) continuous with the ependymal cytoplasm is interpreted as an ependymal secretion product. *C*, cilia; *dc*, dense-cored vesicle; *M*, mitochondria; *m*, microvillus. Three months old rat, ×17,810



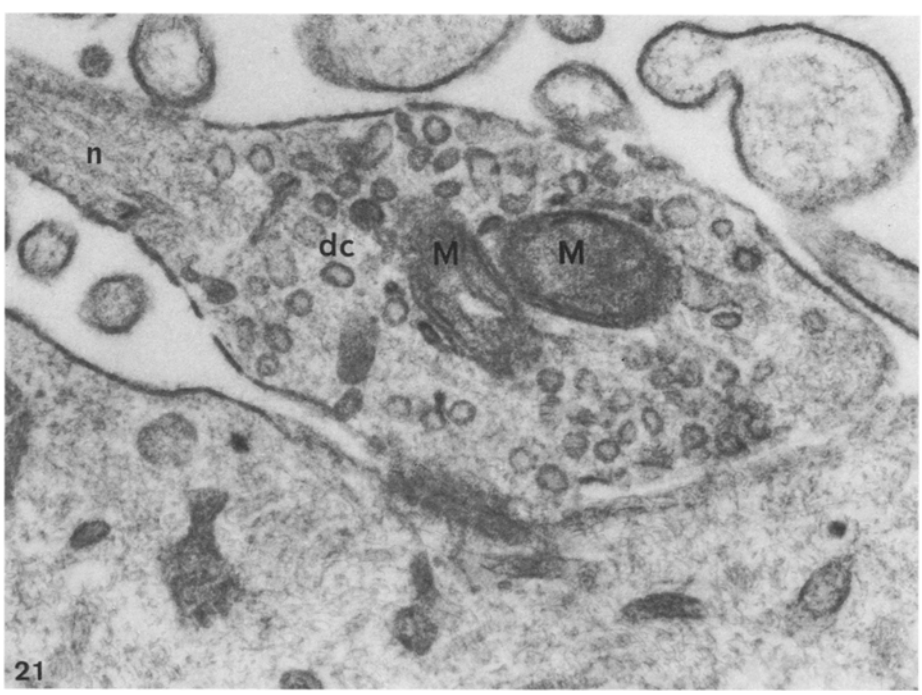


Fig. 20. Desmosome contact between supraependymal axon and ependymal plasmalemma of medial habenular nucleus. *M*, mitochondrium; *m*, microvilli; *s*, synaptic vesicles. Six weeks old rat, ×82,650

especially present on the ventricular floor, mainly in the infundibular recess (Scott *et al.*, 1972, 1973, 1974). The present findings do not allow conclusions about the perikarya of origin of habenular SEA's. According to Aghajanian and Gallager (1975), SEA's in the forebrain and on the rat habenula in particular originate from the midbrain dorsal and median raphe nuclei. In agreement is the finding of Chan-Palay (1976) that a dense population of SEA's in the dorsal aspect of the third ventricle originates mainly from the midbrain raphe. Lorez *et al.* (1975) opine that forebrain SEA's represent branches from serotonergic axons passing through the medial forebrain bundle. Another source of habenular SEA's may be the interpeduncular nuclei, since Ribas (1977) demonstrated degeneration of such fibers following lesion of the interpeduncular nucleus.

The presence of dense-cored vesicles in habenular SEA's may indicate that at least biogenic amines (probably 5-HT) are involved in synaptic transmission between these axons and – as yet not elucidated – postsynaptic elements. A serotonergic nature of rat habenular SEA's previously has been demonstrated using a histofluorescence technique by Lorez and Richards (1973) and by Aghajanian and Gallager (1975). They all made mention of a dense population of terminals. Ribas (1977), in a recent report, also regarded these axons as being indolaminergic in nature. From a detailed study of Chan-Palay (1976), based on autoradiography after intraventricular perfusion of [^{3}H]5-HT in monkeys and rats, it would seem that the entire dorsal third ventricular part is provided with an extensive plexus of serotonergic axons. A similar (serotonergic) (5-HT) nature of forebrain SEA's has been established by Aghajanian and Gallager (1975) and Lorez *et al.* (1975), and of fourth ventricular SEA's by Lorez and Richards (1975).

The presence of numerous small electron-lucent vesicles and scattered large dense-cored vesicles in habenular SEA's conforms with the vesicle population in SEA-terminals as reported for other regions: "lateral ventricle" (Noack *et al.*, 1972), "forebrain" (Lorez *et al.*, 1975), "infundibular recess" (Scott *et al.*, 1973).

The desmosomal nature of the habenular SEA-ependyma junctions conforms to similar specializations reported for axon-ependyma contacts near to the subcommissural organ (Leonhardt and Backhus-Roth, 1969), in the fourth ventricle (Leonhardt and Lindemann, 1973) and lateral ventricle (Noack and Wolff, 1970). Axon-ependyma contacts in the third ventricle have been described as "synaptic-like" by Coates (1973a) and Leonhardt and Backhus-Roth (1969). The axon-ependyma junctions of the rat habenula apparently have features in common with junctions as observed on the rhesus monkey habenula by Kumar and Anand Kumar (1975), *i.e.*, increased density of apposed membranes and frequent occurrence of electron-lucent synaptic vesicles measuring 45–50 nm in diameter.

The results obtained do not elucidate functions of habenular SEA's. According to Chan-Palay (1976), SEA's could be important modifiers of local CSF serotonin content. In respect of the habenula it is noteworthy that both according to Kumar and Anand Kumar (1975, rhesus monkey) and according to Ribas (1977, rat), SEA's may control habenular ependymosecretion. According to Ribas the fibers might exert a maintaining influence upon ependymal cell shape, as would appear from the reduction in height of these cells following lesions of the interpeduncular nucleus initiating habenular SEA-degeneration.

Fig. 21. Desmosome contact between terminal bouton of supraependymal axon and ependyma of medial habenular nucleus. The vesicle population mainly comprises round to oval agranular vesicles. No vesicle aggregation is evident near the membrane thickening. *dc*, dense-cored vesicle; *M*, mitochondria; *n*, neurotubules. Six weeks old rat, ×82,650

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