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Ependymal Specializations

II. Ultrastructural Aspects of the Apical Secretion of the Toad Subcommissural Organ*

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Summary. The ependymal cells of the toad subcommissural organ produce pale and dense secretory granules. Both types of granules are mainly concentrated in the apical cytoplasm and in the perinuclear region. Pale and dense granules are synthesized by and packed in the rough endoplasmic reticulum, bypassing the step of the Golgi apparatus. The apical cytoplasm of some subcommissural ependymal cells protrudes into the ventricle. All the cells project a few cilia and numerous slender, long microvilli into the ventricular lumen.

Contacting the cilia and the microvilli there is a filamentous material identical to that observed in the fibre of Reissner at the aqueduct of Sylvius. In addition to filaments, the fibre of Reissner contains vacuolar formations. The fibre is surrounded by numerous ependymal cilia, some of which are embedded in the filamentous material of the fibre.

The presence of numerous microvilli projected into the ventricle and the large number of vesicles scattered in the supranuclear cytoplasm seem to indicate that the subcommissural organ may have absorption functions. The fact that the intercellular space of the ependymal layer of the subcommissural organ is not separated from the ventricular lumen by tight junctions but by zonulae adhaerentes could indicate that the cerebrospinal fluid penetrates these intercellular spaces bathing all sides of the ependymal cells. The presence in the ependymal cells of vesicles opening into the intercellular space would be in agreement with the latter possibility.

There are some ultrastructural differences between the ependymal cells of the cephalic end of the subcommissural organ and those of the caudal end. A critical analysis of Reissner's fibre formation is made.

Key-Words: Subcommissural organ—Toads—Apical secretion—Fine structure.

The subcommissural organ is one of the most specialized areas of the ventricular walls. Ever since its discovery (Dendy, 1902) it has been the subject of numerous publications and numerous functions have been ascribed to it (for references see: Bugnon, Lenys and Lenys, 1963). However, a new phase in the study of this organ was opened after the demonstration in the ependymal cells of the organ of a substance stainable by the chrome-alum hematoxylin-phloxine method of Gomori (Stutinsky, 1950). Almost simultaneously Mazzi (1952), Bargmann and Schiebler (1952) and Wislocki and Leduc (1952) confirmed the presence of a "Gomori-positive material" in the subcommissural organ cells of

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several species. It was also observed that the ependymal cells of the organ were selectively stained with the periodic acid-Schiff (PAS) reagent (Wislocki and Leduc, 1952; Bargmann and Schiebler, 1952). After these observations, the stainable material was considered to be a secretory product.

The fate of the secretory material of the subcommissural organ has been and still is a source of discussion. Despite the numerous contributions, the data on which the authors base their conclusions are purely morphological in nature. The preferential localization of the secretory material in the apical part of the ependymal cells has led numerous authors to postulate that the material is released into the cerebrospinal fluid (CSF) where, in most cases, it is assumed to condense to form the fibre of Reissner (Wislocki and Leduc, 1954; Wislocki and Roth, 1958; Talanti, 1958; Olsson, 1958; Gomez Bosque, Benito Arranz and Arnaiz, 1958; Oksche, 1961, 1962; Bugnon, Lenys and Lenys, 1963; Hofer, 1967; Sterba, Müller and Naumann, 1967; Naumann, 1968; Leatherland and Dodd, 1968).

On the other hand, it has been postulated that, when the secretory material is mainly localized at the base of the ependymal cells and in their basal processes, the main pathways of release are the blood vessels or the subarachnoid space or both, so that the apical secretion into the CSF would be less important (Oksche, 1954, 1956, 1962; Okada 1955; Murakami, Ban and Aiura, 1957; Campos-Ortega, 1964; Afifi, 1964). After an investigation comprising species from cyclostomes to man, Oksche (1961) explains this differential localization of the secretory material in the subcommissural organ cells in evolutional terms. In lower vertebrates like fishes and amphibians, and, to a lesser degree, birds and reptiles, the secretion is located basally, whereas in the higher forms the secretions are apical and, probably, released mainly into the CSF.

A few ultrastructural observations on the subcommissural organ of amphibians have been reported (Murakami and Tanizaki, 1963; Oksche and Vaupel-von Harnack, 1965; Altner, 1968). The present report describes in detail the ultrastructural organization of the ependymal cells of the toad subcommissural organ and Reissner's fibre. The fine structure of the basal processes of the subcommissural cells and their relationships to hypendymal formations are described in a separate paper (Rodríguez, 1970).

Material and Methods

Sixteen mature male toads *Bufo bufo*, from England, were used. The animals were collected throughout the year and killed within a week after capture. For description of the methods used see Rodríguez (1969).

Results

Light Microscopy. Under the light microscope the subcommissural organ of the toad *Bufo bufo* does not differ from the subcommissural organ of other anuran species (Oksche, 1962) (Figs. 1 and 2). In most cells the stainable material is concentrated at the apical cytoplasm where it forms a thin layer lining the ventricular lumen, and in the infranuclear region. These two areas stain deeply with both aldehyde-fuchsin and PAS. The supranuclear region and the basal processes also show stainable material, but faintly stainable and less abundant.

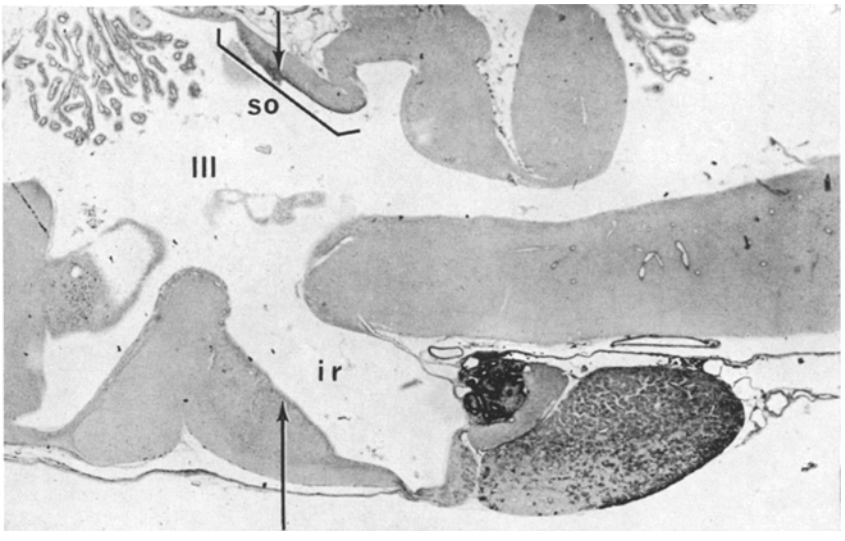


Fig. 1. Mid-sagittal section through the toad brain. The two arrows indicate the level at which the section shown in Fig. 2 was taken. *SO* subcommissural organ; *III* third ventricle; *ir* infundibular recess. Aldehyde fuchsin stain. ×21

There are some minor differences between the caudal and cephalic ends of the organ. The cells of the caudal end always contain a large number of pigment granules, whereas the cells of the cephalic end never show such granules. Moreover, the basal processes of the caudal cells usually end on blood vessels, while those of the cephalic cells end principally on the external limiting membrane bordering the subarachnoid space.

Electron Microscopy. The nuclei of the subcommissural ependymal cells are generally in the basal region of the cells. They are elongated and indented. The chromatin is densely packed against the nuclear envelope. Nucleoli are prominent (Fig. 3). The following regions may be recognized in the subcommissural ependymal cells (Fig. 3):

Apical Surface. Most cells project numerous long, thin microvilli and few cilia into the ventricle. The actual cytoplasm of some cells also protrudes into the ventricle, producing a very irregular apical surface (Figs. 3 and 6). The cilia belong to the 9 + 1 type. Each microvillus contains a few filaments about 5 nm thick, parallel to the major axis of the microvillus (Fig. 4). Apparently lying on the microvilli and cilia there is a filamentous material, which varies in amount from one region of the organ to another. The filaments are about 5 to 7 nm thick (Fig. 4). The same filamentous material is observed in the fibre of Reissner at any point from the aqueduct of Sylvius to the spinal canal. In addition to filaments, the Reissner's fibre shows also large vacuolar and tubular formations, which are three or four times thicker than the ependymal microvilli. The fibre is always partially surrounded by ependymal cilia, and occasionally the cilia are embedded in the fibre (Fig. 5).

Apical Cytoplasm. The main components of this cytoplasmic area are numerous electron dense granules ranging in size from 200 to 400 nm and pale granules of about 130 to 260 nm (Fig. 6). A few transitional forms between the two types of granules may be seen. No secretory granules have ever been observed to open

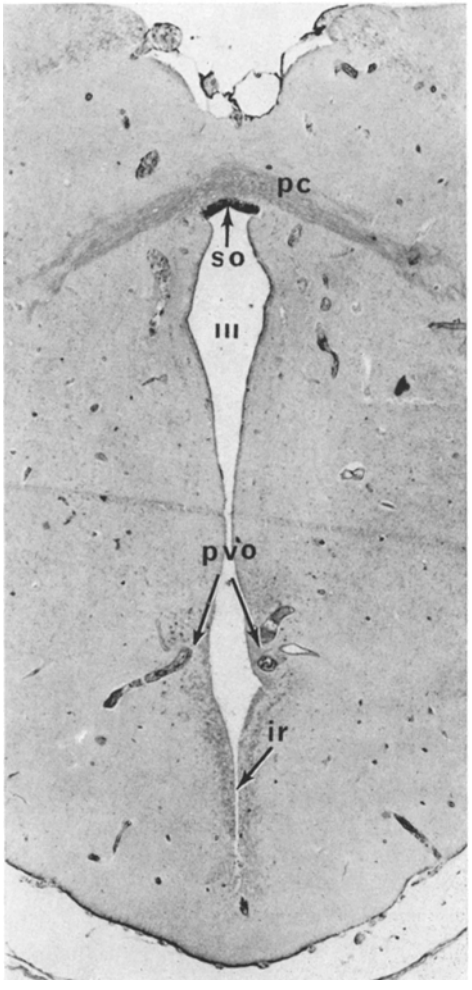


Fig. 2. Frontal section through the diencephalon at the level indicated in Fig. 1. *PC* posterior commissure; *SO* subcommissural organ; *III* third ventricle; *PVO* paraventricular organ; *ir* infundibular recess. Aldehyde fuchsin stain. ×41

directly into the ventricular cavity. Abundant free ribosomes, a few mitochondria, microtubules, centrioles, cilium roots and multivesicular bodies are also ordinary components of the zone.

Supranuclear Cytoplasm. The Golgi complexes are always localized in this particular area of the cytoplasm. Each cell generally contains several of these complexes (Figs. 3 and 7). The membranes lining the Golgi cisternae have a three-layered structure and a thickness of about 5 nm (Fig. 8). No dense material has ever been observed in the lumen of the Golgi cisternae. In addition to the

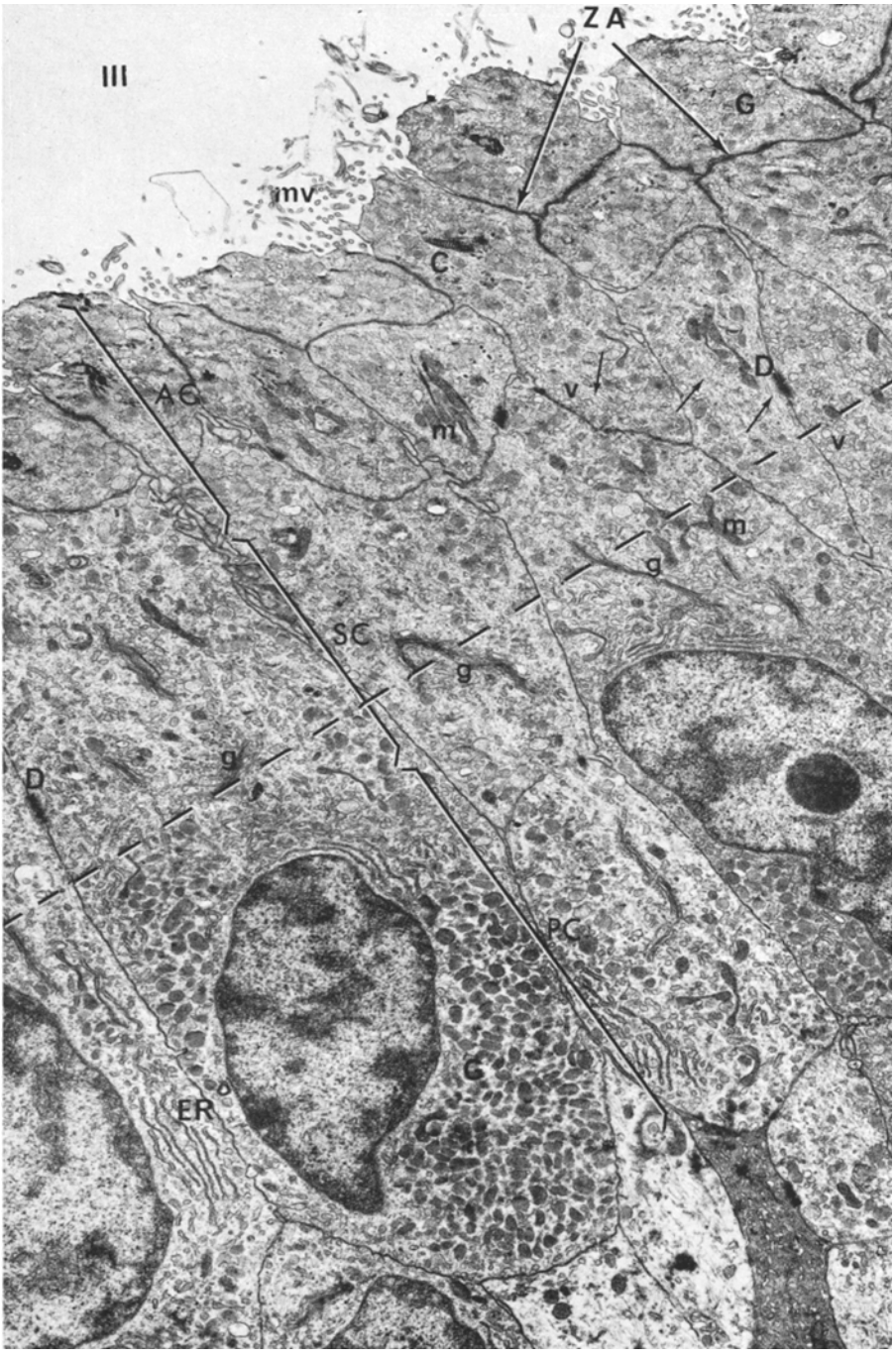
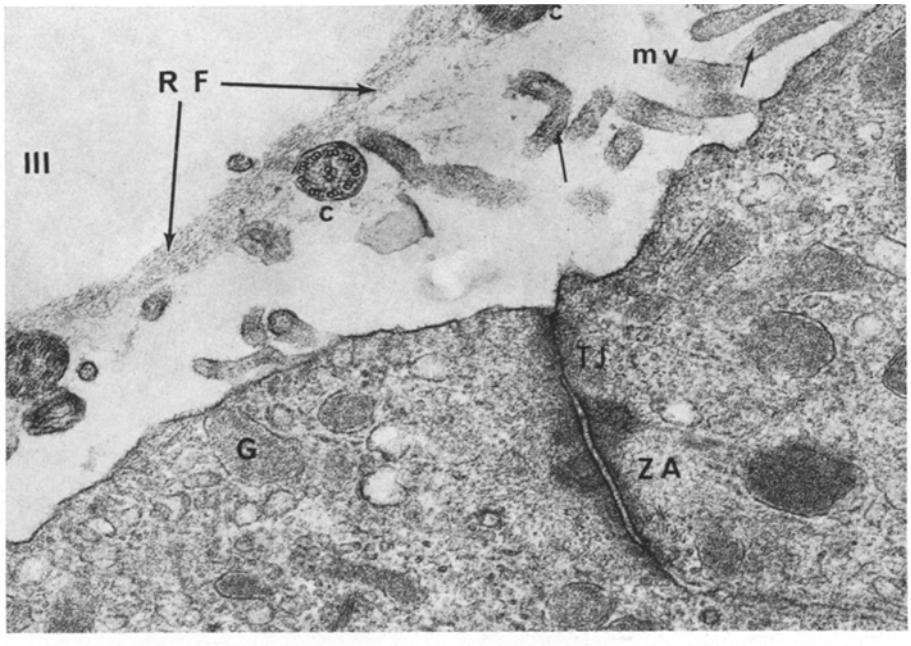


Fig. 3. Ependymal cells of the toad subcommissural organ. *AC* apcial cytoplasm; *SC* supranuclear cytoplasm; *PC* perinuclear cytoplasm; *III* third ventricle; *mv* microvilli; *ZA* zonula adherens; *G* secretory granules; *C* ciliary root; *m* mitochondria; *D* desmosome; *v* vesicles; *g* Golgi complex; *ER* rough endoplasmic reticulum. Small arrows: microtubules. Broken line: approximate plane at which the section shown in Fig. 7 was obtained. ×5,000



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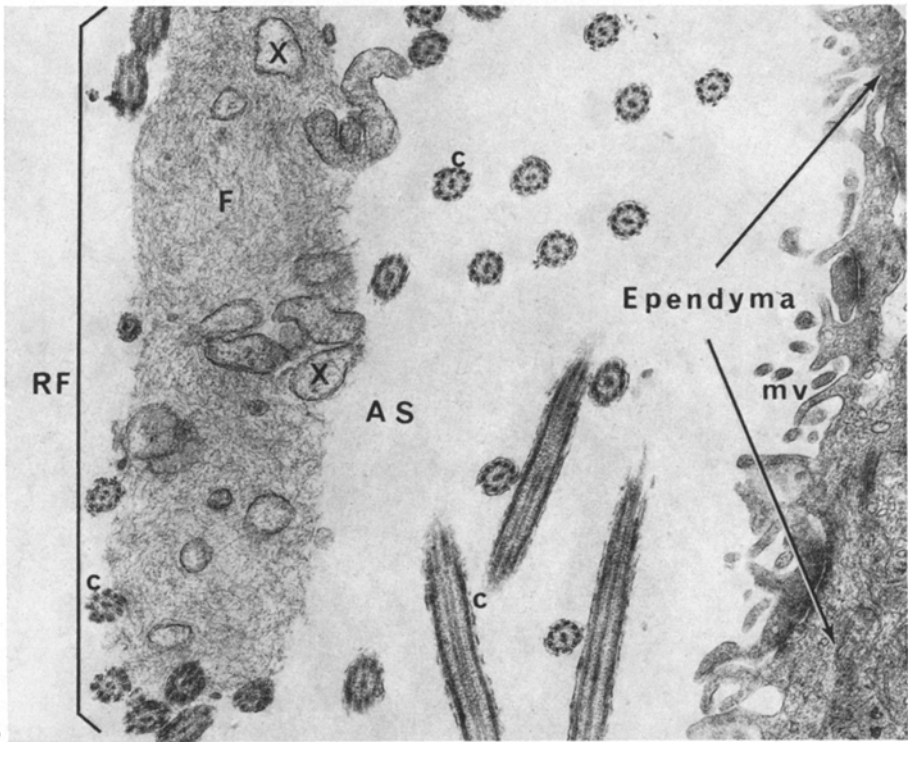

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Figs. 4 and 5

Golgi structures, this area is occupied by mitochondria, a few dense and pale secretory granules, a few cisternae of the rough endoplasmic reticulum, several vesicles of different sizes, and numerous microtubules. As in the case for the microtubules observed in other cells, the wall of these microtubules is formed by filaments and not by a three-layered membrane (Fig. 8).

Perinuclear Cytoplasm. The rough endoplasmic reticulum is particularly well developed in this region. It may be arranged in different ways: the classical disposition of packs of cisternae observed in typical secretory cells; or circular or semi-circular cisternae enclosing numerous ribosomes; or, most commonly, groups formed by a few cisternae intermingled with secretory granules (Fig. 9). Connections between the cisternal membranes and the limiting membrane of the pale granules are frequently seen (Fig. 9). The cisternal lumen is frequently expanded and filled with a material with the same characteristics as that contained in the pale granules. Besides the cisternae of the rough endoplasmic reticulum, the perinuclear cytoplasm contains numerous secretory granules. Pinching-off of granules from the cisternae of the rough endoplasmic reticulum is frequently seen. Intermediate forms between the small and pale granules pinched-off from the cisternae and the dense and large granules are frequent in the neighbourhood of the rough endoplasmic reticulum (Figs. 9 and 10). The pale granules contain a filamentous material, whereas the dense granules are filled with a fine granular and microvesicular material (Figs. 4 and 10).

The limiting membrane of the granules is a three-layered structure. Most granules are elongated with a width ranging between 100 and 300 nm and a length between 200 and 800 nm. Scarce mitochondria, multivesicular bodies, lysosome-like bodies and microtubules are also seen in this area of the subcommissural cells.

Spinous surfaced vesicles occasionally open into the thin intercellular space (Fig. 9).

Basal Processes. These formations are described in a separate paper (Rodríguez, 1970).

Cell Junctions. The very end of the apical portion of the intercellular space is sealed by zonulae adhaerentes (nomenclature proposed by Brightman and Palay, 1963), which form an uninterrupted barrier between the ventricular lumen and the intercellular spaces (Figs. 3 and 6). Occasionally a tight junction is interposed between the zonula adhaerens and the free surface of the cells. Underneath the zonula adhaerens the cells interdigitate more or less extensively (Figs. 6 and 7). Desmosome junctions are numerous and are observed between the cell bodies,

Fig. 4. Apical cytoplasm and free surface of two subcommissural ependymal cells. *III* third ventricle; *C* cilia; *RF* filaments of the Reissner's fibre; *mv* microvilli; *G* secretory granules containing microvesicles; *TJ* tight junction; *ZA* zonula adherens. Small arrows: thin filaments within microvilli. ×34,000

Fig. 5. Cross section through the fibre of Reissner's (*RF*) at the level of the aqueduct of Sylvius (*AS*). *F* filaments of the Reissner's fibre; *X* vacuolar formations embedded in the fibre; *C* cilia; *mv* microvilli of the ependymal cells lining the floor of the aqueduct of Sylvius. ×20,000

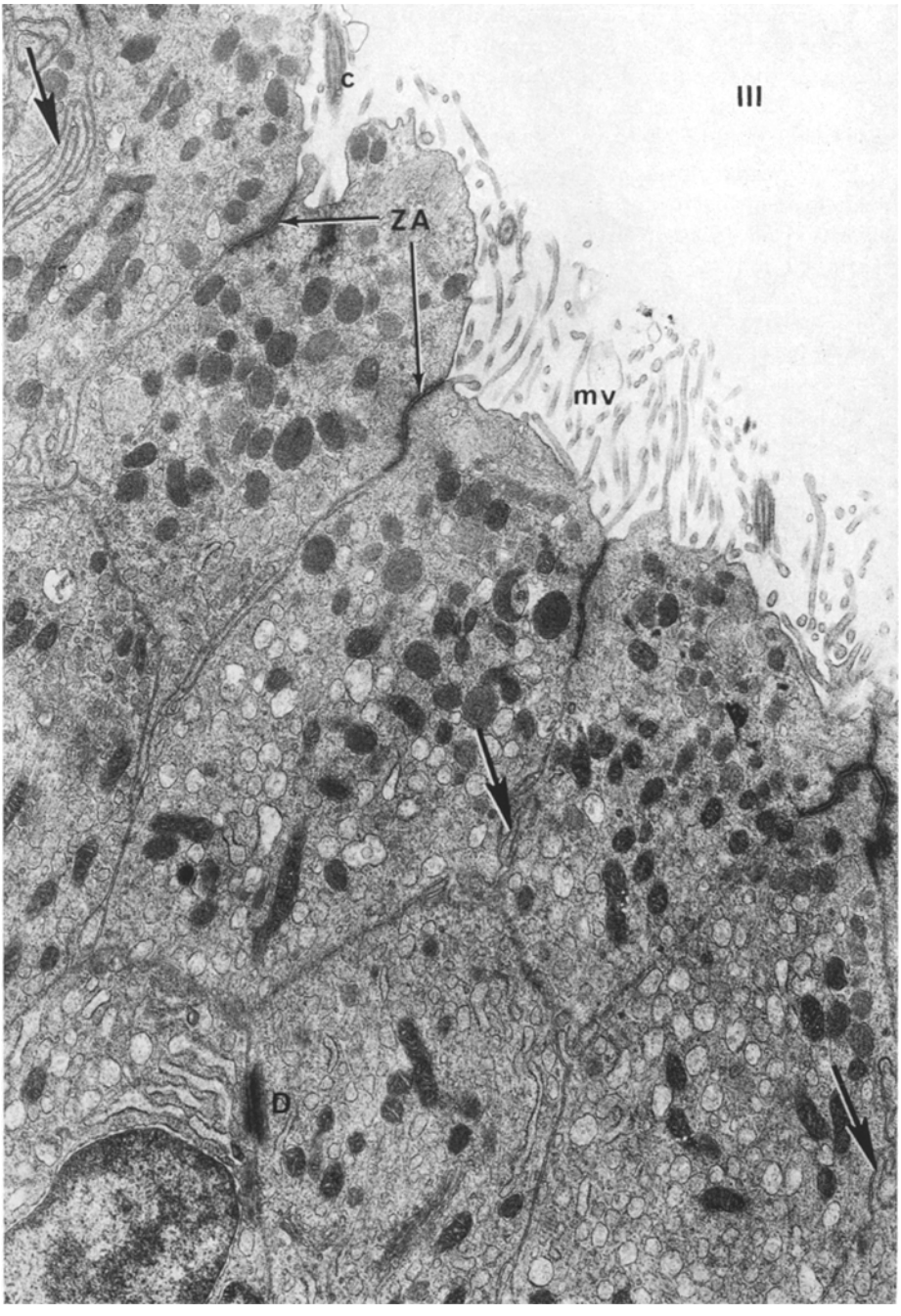

Fig. 6. Apical cytoplasm of subcommissural ependymal cells containing numerous pale and dense secretory granules. *III* third ventricle; *c* cilia; *mv* microvilli; *ZA* zonula adherens; *D* desmosome. Large arrows: interdigitations of the ependymal cells. ×12,000

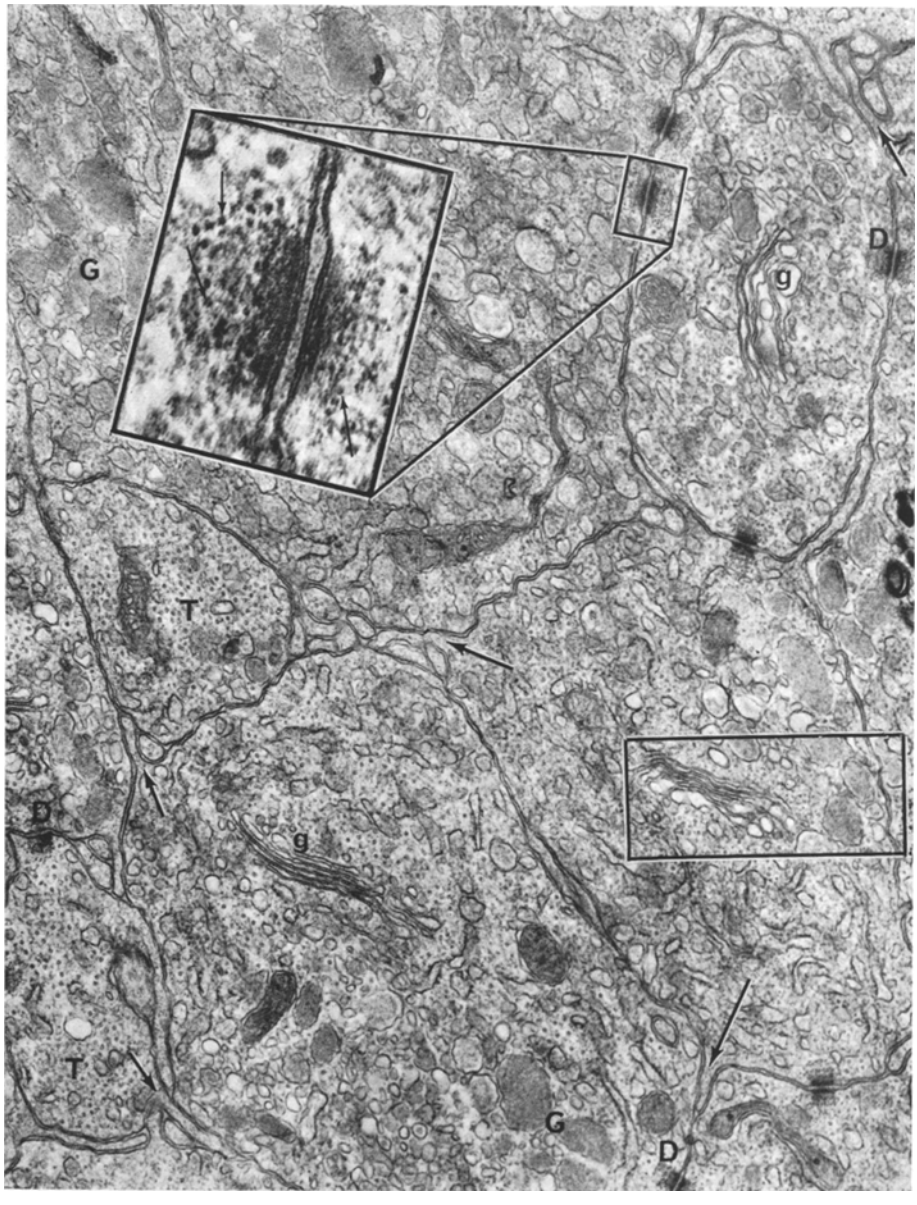

Fig. 7. Horizontal section of subcommissural ependymal cells at the level of the supranuclear cytoplasm (for orientation see Fig. 3). Note the presence of numerous desmosomes (*D*), Golgi complexes (*g*), microtubules (*T*) and lateral interdigitations (arrows). *G* secretory granules. The area enclosed in the rectangle at right is shown at higher mangification in Fig. 8. ×24,000. *Insert.* Desmosome showing the trilaminar structure of the cell membrane and the microtubular appearance of the desmosomal filaments (small arrows). ×110,000

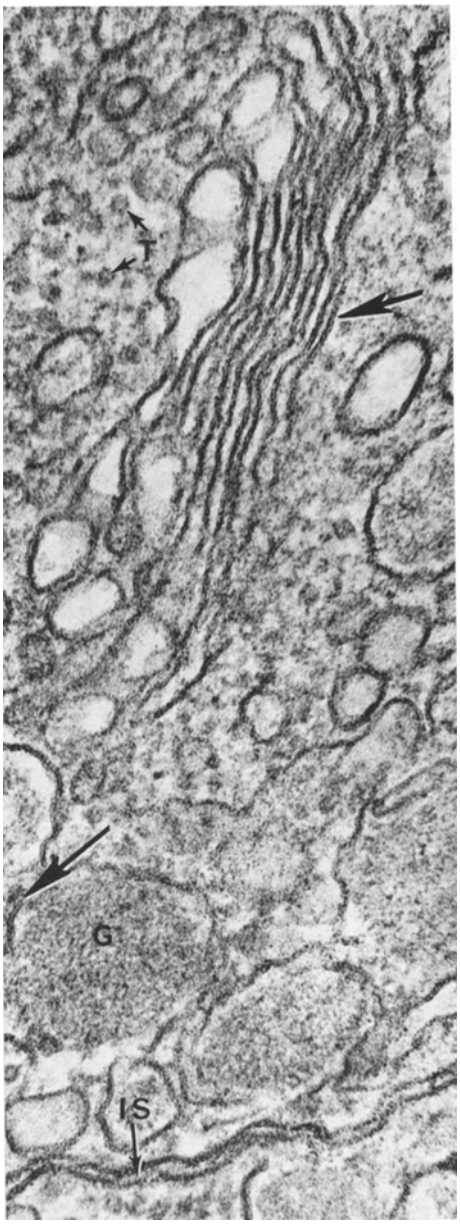

Fig. 8. High magnification of the area framed in rectangle of Fig. 7. Large arrows: trilaminar structure of the Golgi membranes and the limiting membrane of the secretory granule (*G*). *T* microtubules; *IS* intercellular space. ×110,000

the paired basal processes and also between the endings contacting the external limiting membrane (Fig. 3).

Ependymal Cell of the Caudal End. These cells show some characteristics which clearly differentiate them from the remaining subcommissural ependymal cells. The infranuclear region is mainly occupied by an onion-like arrangement of

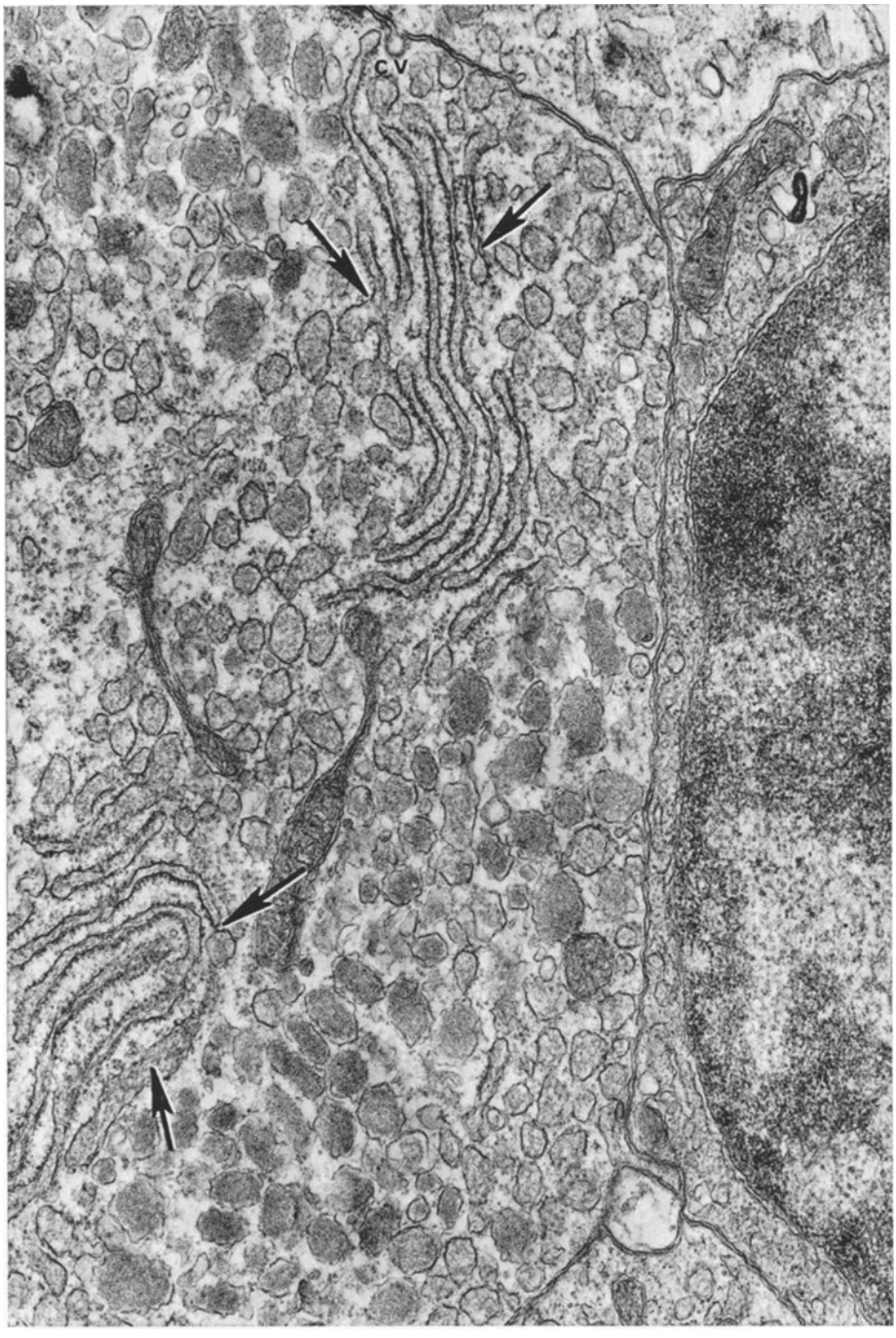

Fig. 9. Perinuclear region of four ependymal cells showing numerous rough endoplasmic reticulum cisternae and secretory granules of different sizes and densities. Arrows: pinching-off of secretory granules from cisternae of the endoplasmic reticulum. *cv* coated vesicle opening into the intercellular space. ×30,000

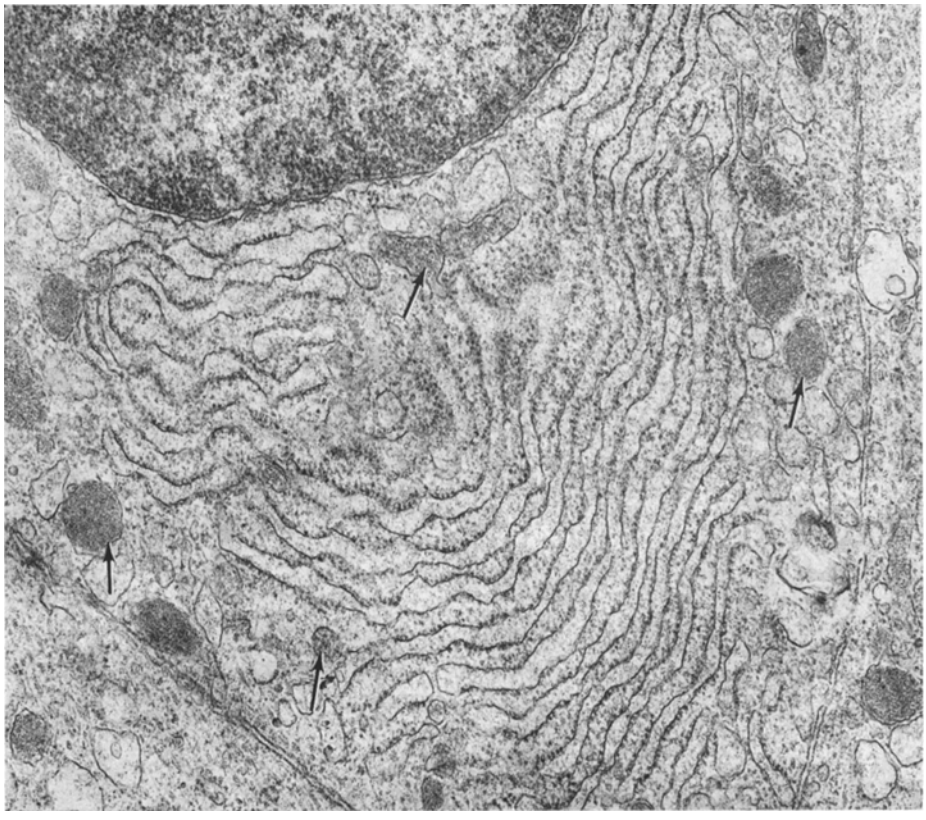

Fig. 10. Infranuclear region of a subcommissural ependymal cell showing a well developed rough endoplasmic reticulum. Arrows: granules with a microvesicular content. ×27,500

concentrical cisternae of the rough endoplasmic reticulum. Pigment-like bodies are abundant in these cells. The secretory granules are much less numerous in the cells of the caudal end of the organ than in those of the cephalic portion (Fig. 11).

Discussion

Although most who have studied the subcommissural organ-Reissner's fibre complex during the past twenty years agree that the Reissner's fibre is a secretory product of the subcommissural organ, there seem to be no conclusive proofs in favour of this hypothesis. Most observations supporting this hypothesis are based on morphological studies. After Stutinsky (1950) had shown that both the subcommissural organ and the Reissner's fibre were stained by the aldehyde-fuchsin technique, numerous authors have confirmed this observation in all species studied (for references see: Talanti, 1958; Oksche, 1961). During the past few years more sophisticated techniques have confirmed that the secretions of the subcommissural organ and the Reissner's fibre share the same histochemical properties (Sterba *et al.*, 1967; Naumann, 1968). Sterba (1967) and Leatherland and Dodd (1968) have

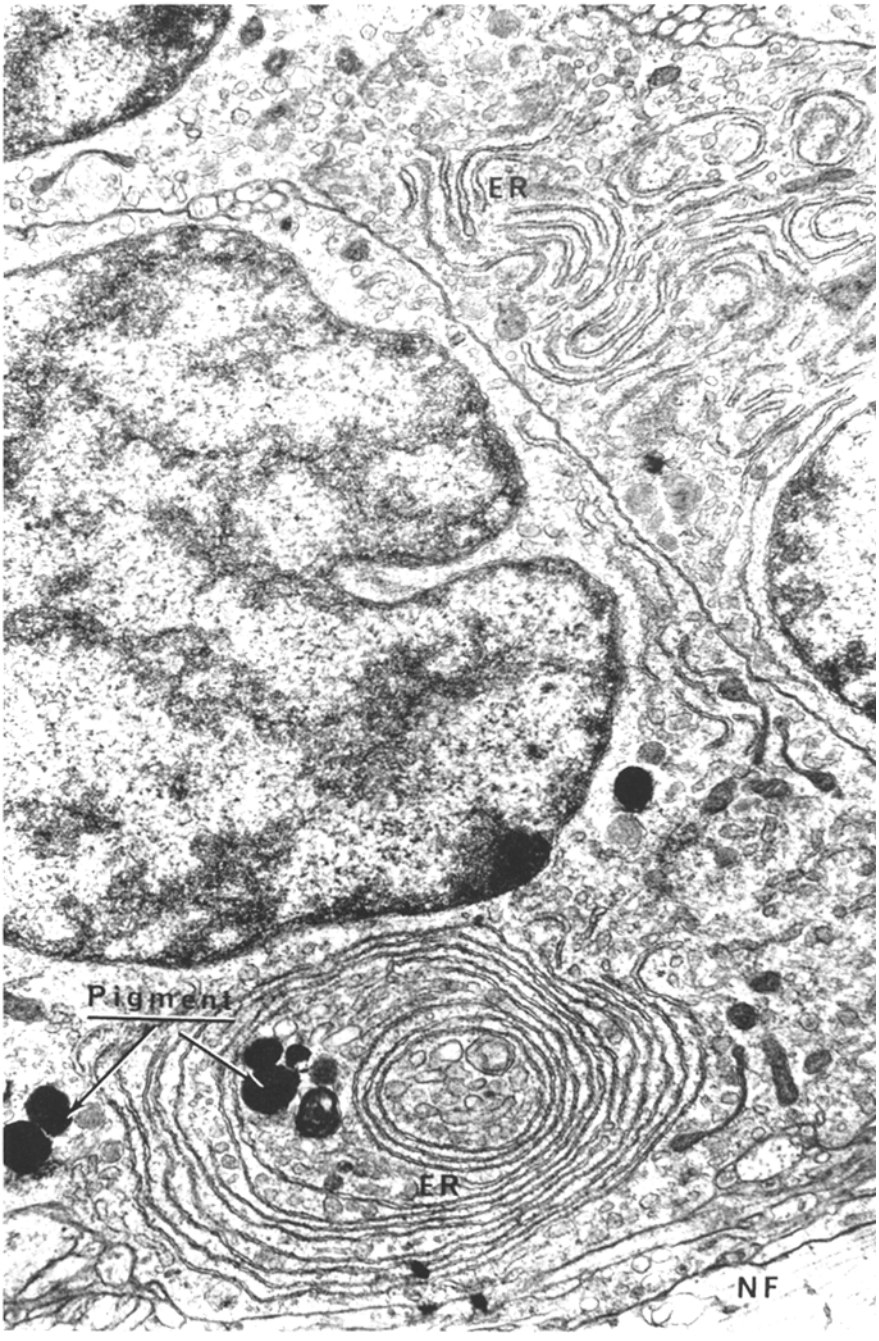

Fig. 11. Ependymal cells of the caudal end of the subcommissural organ. In one of the cells the cisternae of the rough endoplasmic reticulum (*ER*) are concentrically arranged. *NF* subependymal nerve fibre. ×15,000

reported that the ependymal cells of the subcommissural organ as well as the fibre of Reissner incorporate and concentrate intraventricularly injected S^{35} cysteine.

The electron microscopists have also presented some evidence supporting the subcommissural origin of the Reissner's fibre. Thus, Sterba *et al.* (1967) postulated that the secretory material contained in the dilated cisternae of the rough endoplasmic reticulum is transported by tubular formations to the free surface of the ependymal cells, where it is released into the cerebrospinal fluid (CSF) and aggregates to form the Reissner's fibre. They based their hypothesis only on the ultrastructural appearance of the secretory material, which they claim to be similar in the three compartments, rough endoplasmic reticulum cisternae, tubules and ventricle. However, critical analysis of the electron micrographs published by these authors clearly shows that the content of the apical tubules is different from that of the endoplasmic reticulum and from the flocculated material present in the ventricle. The presence in the ventricular lumen of ependymal protrusions and of "isolated" cytoplasmic masses has led some authors to speak of an apocrine or merocrine secretion of the subcommissural organ, which would be responsible for the production of the Reissner's fibre (Murakami and Tanisaki, 1963; Leatherland and Dodd, 1968; Papacharalampous *et al.*, 1968). However, it seems obvious that the ependymal protrusions as such cannot be regarded as an apocrine secretion. The "isolated" ependymal masses could be regarded as an apocrine secretion only if they were shown to be lying free in the ventricular lumen through serial section studies. Since such studies have not been carried out, apocrine secretion of the subcommissural organ can only be regarded as a hypothesis.

Other authors have postulated that the secretory material of the subcommisural ependymal cells is excreted into the third ventricle by fusion of the granule membrane with the apical cell membrane (Murakami and Tanisaki, 1963; Stanka *et al.*, 1964; Isomäki *et al.*, 1965; Altner, 1968). However, only figure seven of Stanka's *et al.* paper shows a secretory granule distinctly opening into the ventricular lumen. The question is whether these occasional connections between secretory granules and ventricle represent the normal or main mechanism for the excretion into the ventricle of the subcommissural secretory product(s).

Evidence supporting either aprocrine secretion or secretion by "exocytosis" has not been found in the present study. In the toad, the ultrastructural appearances of the content of the secretory granules and of the Reissner's fibre differ considerably.

In summary, after analyzing most of the bibliography on Reissner's fibre formation, it seems justified to call attention upon the fact that conclusive evidence of the subcommissural origin of the Reissner's fibre has, so far, not been presented. The facts that the subcommissural ependymal cells and the fibre of Reissner share the same histological and histochemical properties and in some species the same ultrastructural appearance, that one of the ends of the fibre is "attached" to the subcommissural organ, and that development of the Reissner's fibre stops after destruction of the subcommissural organ (Olsson, 1958) do not seem to be *conclusive enough* as to regard the subcommissural origin of the fibre as an unquestionable fact.

The apparent absence of Reissner's fibre in some species (Murakami *et al.*, 1957, 1963; Wislocki and Roth, 1958; Talanti, 1959) which do possess a well developed subcommissural organ could indicate that the main function of the organ is not the production of the Reissner's fibre. However, it is very likely that the fibre of Reissner is present in all species and that its absence from the histological preparations is due to a defective fixation. Rodríguez (1969) has shown that the best way to fix the Reissner's fibre is to perfuse the cerebral ventricles with aldehydes at a given pressure.

The hypertension of the perfusate causes the detachment of the fibre. A fibre "loose" within the ventricles can easily be washed out by the fixing and dehydrating solutions. If the brain is fixed by vascular perfusion the Reissner's fibre remains loosely attached to the ventricular walls, including the subcommissural organ, and it may also be removed from the ventricles during any of the steps that follow the perfusion process. Therefore, the fixation of the fibre of Reissner in the position it occupies *in vivo* seems to be rather tricky; the absence of the fibre from the histological preparations does not necessarily mean, thus, that the fibre is really missing in the living animal.

The presence within the fibre of membranous structures seems to be a common phenomenon (Müller and Sterba, 1965; Sterba *et al.*, 1967; Altner, 1968; Kohno, 1969). The fact that in the toad these structures are considerably larger than the ependymal microvilli would rule out the possibility that they are microvilli embedded within the fibre, as postulated by Sterba *et al.* (1967) for the lamprey. Our observations agree with those of Kohno (1969) concerning the filamentous structure of the Reissner's fibre. However, unlike the rat Reissner's fibre, the toad fibre is not formed by parallel filaments, but by whirled filaments. In the toad the filaments of the Reissner's fibre may be parallel only in the region of the subcommissural organ. The close spatial relationship between Reissner's fibre and ependymal cell cilia is striking, especially at the aqueduct of Sylvius.

Another interesting question with respect to the function of the subcommissural organ is whether the "apical" or "exocrine" secretion of the organ is concerned only with the formation of the Reissner's fibre or if the organ also secretes into the ventricle some principles not related to the Reissner's fibre. The presence of two types of secretory granules in the apical cytoplasm of the toad subcommissural cells would support the latter possibility. Pale and dense granules similar to the ones observed in the toad have been found in the subcommissural organ of several species (Murakami and Tanisaki, 1963; Papacharalampous *et al.*, 1968; Oksche, 1969). However, there is no evidence suggesting that the two types of granules represent two types of secretory products. This seems to be an interesting task for future investigations. The present study shows that both types of secretory granules are formed in the rough endoplasmic reticulum. This is in agreement with Oksche's observations (1969) in the dog but disagrees with the observations of Murakami and Tanisaki (1963) in the toad and those of Papacharalampous *et al.* (1969) in the guinea pig. According to these two groups of authors the dense granules are formed in the Golgi apparatus and the pale granules in the rough endoplasmic reticulum. The fact that the secretory mechanism which results in the production of the secretory granules of the subcommissural cells bypasses

the step of the Golgi complex is in itself a remarkable feature and certainly deserves to be further investigated.

The subcommissural organ should not be regarded only as a secretory organ but also as a formation capable of absorption. The relatively large number of regularly shaped microvilli protruding into the ventricle and the numerous vesicles scattered in the supranuclear cytoplasm are two features indicative of absorptive properties. Löfgren (1965) has shown that the subcommissural organ can absorb intraventricularly injected dyes. The lack of an uninterrupted band of tight junctions sealing the apical margins of the subcommissural cells could indicate that the intercellular spaces of this part of the ventricular walls are permeable to the CSF. The presence in these ependymal cells of coated vesicles opening into the intercellular space would indicate that these cells may absorb or secrete substances from or into the intercellular space.

The zonulae adhaerentes form a thick and continous "belt" around the apical edge of the subcommissural ependymal cells. This and the presence of numerous desmosome junctions are two distinctive features of this highly specialized ependyma.

The fact that the ependymal cells lining the caudal end of the subcommissural organ have some particular characteristics, such as the presence of pigment inclusions and of flat cisternae of rough endoplasmic reticulum concentrically arranged, could indicate that in the subcommissural organ there is some kind of zonation.

References

Afifi, A. K.: The subcommissural organ of the camel. J. comp. Neurol. **123**, 139–145 (1964).

Altner, H.: Untersuchungen an Ependym und Ependymorganen im Zwischenhirn niederer Wirbeltiere (*Neoceratodus*, Urodelen, Anuren). Z. Zellforsch. **84**, 102–140 (1968).

Bargmann, W., Schiebler, T. H.: Histologische und cytochemische Untersuchungen am Subcommissuralorgan von Säugern. Z. Zellforsch. **37**, 583–596 (1952).

Brightman, M. W., Palay, S. L.: The fine structure of ependyma in the brain of the rat. J. Cell Biol. **19**, 415–439 (1963).

Bugnon, C., Lenys, R., Lenys, D.: Quelques aspects particuliers de l'organe sous-commissural du Rat blanc et quelques observations sur l'organe sous-commissural du Lapin, du Porc et du Cheval. Ann. Sci. Univ. Besançon, 2e Série, Méd. **7**, 61–80 (1963).

Campos-Ortega, J. A.: Aportaciones al estudio histológico del órgano subcomisural del *Lebistes reticulatus* (Peters). Arch. esp. Morfol. **18**, 209–227 (1964).

Dendy, A.: On a pair of ciliated grooves in the brain of the ammocoete, apparently serving to promote the circulation of the fluid in the brain cavity. Proc. roy. Soc. Serie B **69**, 485–494 (1902).

Gomez-Bosque, P., Benito Arranz, S., Arnaiz, S.: L'organe sous-commissural chez *Cavia cobaya* adulte. Acta anat. (Basel) **33**, 65–75 (1958).

Hofer, H.: Beobachtungen an dem sogenannten „Supracommissuralen Organ" (Fuse) und am Recessus mesocoelicus der Primaten. Folia primat. **5**, 190–200 (1967).

Isomäki, A. M., Kivalo, E., Talanti, S.: Electron-microscopic structure of the subcommissural organ in the calf (*Bos taurus*) with special references to secretory phenomena. Ann. Acad. Sci. fenn. A 5 **111**, 3–64 (1965).

Kohno, K.: Electron microscopic studies on Reissner's fiber and the ependymal cells in the spinal cord of the rat. Z. Zellforsch. **94**, 565–573 (1969).

Leatherland, J. F., Dodd, J. M.: Studies on the structure, ultrastructure and function of the subcommissural organ-Reissner's fibre complex of the european eel *Anguilla anguilla* L. Z. Zellforsch. **89**, 533–549 (1968).

Löfgren, F.: On an absorptive ability of the subcommissural organ. A model test. Acta Univ. Lund, Sect. II, No **29**, 3–12 (1965).
Mazzi, V.: Caratteri secretori nelle cellule dell' organo sottocommissurale dei Vertebrati inferiori. Arch. Zool. ital. **37**, 445–464 (1952).
Müller, H., Sterba, G.: Elektronenmikroskopische Untersuchungen des Subcommissuralorgans von *Lampetra planeri* (Bloch). Zool. Anz., Suppl. **29**, 441–453 (1966).
Murakami, M., Ban, F., Aiura, S.: Histologische Studien des Subcommissuralorgans des *Gecko japonicus*. Kurume med. J. **4**, 8–17 (1957).
— Tanisaki, T.: An electron microscopic study on the toad subcommissural organ. Arch. Histol. Japon. **23**, 337–358 (1963).
Naumann, W.: Histologische Untersuchungen am Subcommissuralorgan und am Reissnerschen Faden von *Lampetra planeri* (Bloch). Z. Zellforsch. **87**, 571–591 (1968).
Okada, M.: On the secretory pathway of the subcommissural organ. Arch. histol. Japon. **9**, 199–204 (1955).
Oksche, A.: Über die Art und Bedeutung sekretorischer Zelltätigkeit in der Zirbel und im Subcommissuralorgan. Verh. anat. Ges. (Jena) **52**, 88–96 (1954).
— Funktionelle histologische Untersuchungen über die Organe des Zwischenhirndaches der Chordaten. Anat. Anz. **102**, 404–419 (1956).
— Vergleichende Untersuchungen über die sekretorische Aktivität des Subkommissuralorgans und den Gliacharakter seiner Zellen. Z. Zellforsch. **54**, 549–612 (1961).
— Histologische, histochemische und experimentelle Studien am Subkommissuralorgan von Anuren (mit Hinweisen auf den Epiphysenkomplex). Z. Zellforsch. **57**, 240–326 (1962).
— The subcommissural organ. J. Neuro-Visc. Relat., Suppl. **2**, 111–139 (1969).
— Vaupel-von Harnack, M.: Elektronenmikroskopische Untersuchungen an den Nervenbahnen des Pinealkomplexes von *Rana esculenta* L. Z. Zellforsch. **68**, 389–426 (1965).
Olsson, R.: The subcommissural organ. Thesis. Stockholm 1958.
Papacharalampous, N. X., Schwink, A., Wetzstein, R.: Elektronenmikroskopische Untersuchungen am Subcommissuralorgan des Meerschweinchens. Z. Zellforsch. **90**, 202–229 (1968).
Rodríguez, E. M.: Fixation of the central nervous system by perfusion of the cerebral ventricles with a threefold aldehyde mixture. Brain Res. **15**, 395–412 (1969).
— Ependymal specialization III. Ultrastructural aspects of the basal secretion of the toad subcommissural organ. Z. Zellforsch. **111**, 32—50 (1970).
Stanka, P., Schwink, A., Wetzstein, R.: Elektronenmikroskopische Untersuchungen des Subkommissuralorgans der Ratte. Z. Zellforsch. **63**, 277–301 (1964).
Sterba, G.: Incorporation of sulphur-35 into the subcommissural organ and Reissner's fibre in *Cyprinus carpio*. Nature (Lond.) **216**, 504 (1967).
— Müller, H., Naumann, W.: Fluoreszenz- und elektronenmikroskopische Untersuchungen über die Bildung des Reissnerschen Fadens bei *Lampetra planeri* (Bloch). Z. Zellforsch. **76**, 355–376 (1967).
Stutinsky, F.: Colloide, corps de Herring et substance Gomori positive de la neurohypophyse. C.R. Soc. Biol. (Paris) **144**, 1357–1360 (1950).
Talanti, S.: Studies on the subcommissural organ in some domestic animals. Ann. Med. exp. Fenn. **36**, Suppl. No 9, 1–97 (1958).
— Studies on the subcommissural organ of the bovine fetus. Anat. Rec. **134**, 473–490 (1959).
Wislocki, G. B., Leduc, E. H.: The cytology and histochemistry of the subcommissural organ and Reissner's fiber in the rodents. J. comp. Neurol. **97**, 515–544 (1952).
— — The cytology of the subcommissural organ, Reissner's fiber, periventricular glial cells and posterior collicular recess of the rat's brain. J. comp. Neurol. **101**, 283–309 (1954).
— Roth, W. D.: Selective staining of the human subcommissural organ. Anat. Rec. **130**, 125–133 (1958).

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