The Fine Structure of the Follicle Gland of the Snail, *Lymnaea auricularia*

(Mollusca: Gastropoda)

BY

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(Plates 1 to 4)

INTRODUCTION

THE FOLLICLE GLAND of the cerebral ganglion of pulmonate gastropods was first described by DE NABIAS (1898) and by PELSENEER (1901). It has recently been reinvestigated by LEVER and his colleagues in Amsterdam (LEVER, 1958; LEVER *et al.*, 1959; LEVER and JOOSSE, 1961) in several basommatophoran and stylommatophoran species. On the basis of light-microscope observations, LEVER has raised the possibility that the follicle gland is a neuroendocrine organ with neurosecretory activities. WAUTIER *et al.* (1961) consider the gland in *Gundlachia* as devoted exclusively to secretion. In *Arion,* VAN MOL (1960) describes an equivalent structure as a non-neural endocrine organ (the "cephalic gland"), and this opinion is apparently shared by NOLTE (1963), who terms a similar organ "cerebral gland" in the Helicidae. However, an embryonic vesicle giving rise to neurons but disappearing in later development is also described by SANCHEZ & BORD (1958) in *Helix aspersa.* Whether all these cerebral vesicles represent the same entity is difficult to decide.

The present study of the ultrastructure of the follicle gland was undertaken initially in the hope of finding a simple neurosecretory system, spatially restricted in scope, in which the formation, transport, and release of neurosecretion could be studied in neurons with short axonal processes. In addition, the gross structural similarity of this gland to the epistellar body of the octopus and to the parolfactory vesicles of squids has raised the possibility that mollusks generally might possess a family of related vesicular organs associated with the nervous system (*cf.* NISHIOKA *et al.*, 1962; BERN & HAGADORN, 1964).

MATERIALS AND METHODS

The adult freshwater snails, *Lymnaea auricularia* (identified by Dr. R. Stohler), used in this study were collected from Grayson Creek near Concord, California. The specimens used for light microscopy were fixed in Stieve's and Helly's fixatives, embedded in paraffin, sectioned at 5μ and stained with paraldehyde fuchsin. The animals used for electron-microscope observations were fixed in 1% osmium tetroxide buffered with veronal-acetate (pH 7.5) and embedded in Maraglas-Cardolite (FREEMAN & SPURLOCK, 1962). Thin sections were cut on a Porter-Blum microtome, picked up on formvar-coated copper grids backed with carbon, stained with uranium acetate (WATSON, 1958) and lead citrate (REYNOLDS, 1963), and examined in a RCA EMU 3G electron microscope.

OBSERVATIONS

The follicle gland is located laterally in the lateral lobes of the cerebral ganglion, adjacent to the epineurial capsule. The follicle itself consists of a single layer of low columnar epithelioid cells surrounding an ovoid lumen (Figure 1). In adult specimens, the follicle has an average diameter of about 30μ, and its epithelial wall is about 10μ thick.

Paraldehyde fuchsin-positive colloidal material is present within the lumen. Most of this material is generally found just apical to the follicle cells. The presence of a low, irregular brush-border is discernible on the follicle cells in areas where only a small amount of colloid substance is present.

There were no neurons filled with paraldehyde fuchsin-positive droplets in the lateral lobe of *Lymnaea auricularia,* as have been described by LEVER (1958), LEVER *et al.* (1959), and LEVER & JOOSSE (1961) in several species of Basommatophora, including *L. stagnalis.* Only a haze of fuchsinophilic material was observed in a few neuron perikarya and axons. As a result neuronal processes were difficult to trace with certainty in our preparations. Most

of the nerve processes of the lateral lobe neurons appear to enter the neuropil of the adjacent cerebral lobe. Other processes were directed toward the follicle, but it could not be determined whether they terminated in association with the follicle.

Ultrastructural observations failed to establish the presence of neurosecretory material in the lumen of the follicle gland. Instead, the lumen is occupied by randomly-oriented microvilli and cilia, except for a small, clear central area (Figures 2 and 3). The branching microvilli extend from the apices of the epithelioid cells to form a dense network. Intermingled with the microvilli are cilia, which extend into the lumen from the processes of less common neuron-like cells that also contribute to the follicle wall.

The cytoplasm of the epithelioid cells is dense, except at the apical margin, and contains many ribosomes, some of which are associated with cisternae of the endoplasmic reticulum (Figures 4 and 5). The mitochondria and Golgi apparatus appear typical in form. Some multivesicular bodies and lysosome-like bodies are also present. The nuclei are generally ovoid, oriented perpendicular to the capsule.

The processes of the neuron-like cells can be distinguished from the cytoplasm of epithelioid cells owing to their lack of density (Figures 2 to 5). A few ribosomes are found in the cytoplasm, in addition to some small vesicles of variable size. The mitochondria are large and irregular in shape. The cristae are distantly spaced, thereby giving the mitochondria an empty appearance (Figures 4 and 5). The nuclei of the neuron-like cells are larger than those of the epithelioid cells. Some of the former cells are present within the capsule surrounding the follicle; the perikarya of others are presumably located outside the capsule (Figure 3).

The neurons present in the small area of the lateral lobe adjacent to the follicle were cursorily examined in the electron microscope. These neurons possessed the usual organelles, and some also contained numerous vesicles of assorted sizes. Certain of these neurons contained mitochondria resembling those found in the processes of the follicle gland. No typical elementary neurosecretory granules (electron-dense, in the 1000 to 3000Å range) were found in any of the neurons examined in the lateral lobe. However, many neurons with typical neurosecretory granules have been encountered in the cerebral ganglion proper.

DISCUSSION

The follicle gland of *Lymnaea auricularia* superficially has a neuroendocrine appearance at the light-microscope level. Lever (1958), Lever *et al.* (1959), and Lever & Joosse (1961) have studied this structure in several species of Basommatophora and Stylommatophora and have reported that an occasional bipolar neuron sends a process into the lumen; the lumen is often filled with densely-staining material, which they assume is neurosecretion.

On the electron-microscope level no electron-dense neurosecretory granules could be found in the follicle gland or in the lateral lobe of the cerebral ganglion in which it is located. The fuchsinophilia of the follicle contents is ascribed to accumulations of microvilli. The fuchsinophilic "secretion" of the octopus epistellar body also proved to be stacks of microvilli (Nishioka *et al.*, 1962).

It is of interest that de Nabias (1898) described the presence of cilia in the lumen of the follicle gland, which were attached to processes of the surrounding bipolar cells. To this pioneer investigator, the neuron-epithelium relation in the follicle gland was reminiscent of that seen in the vertebrate olfactory receptor.

It is conceivable that the masses of randomly arranged microvilli of the follicle gland located within the "brain" could reflect a former photoreceptive function of this structure, in an ontogenetic or a phylogenetic sense. Studies of the eye of two land gastropods, *Helix aspersa* (Eakin, 1963) and *Helix pomatia* (Röhlich & Török, 1963), have shown that their photoreceptors are rhabdomeric in type, composed of microvilli radially arranged on the sensory cell process. Although the molluscan photoreceptor is typically rhabdomeric, Clark (1963) has reported that the eye of *Viviparus maleatus* is of the ciliary type. Accordingly, the presence of cilia on the presumably neuronal processes extending into the lumen could also be in accord with a possible photoreceptive structure. However, it would be too facile to suggest that all closed vesicles, such as the follicle gland, associated with the protostome nervous system, are vestigial photoreceptors. In fact, the persistent follicles in gastropods are remnants of the embryonic cerebral tubes, at one time connected to the exterior. Some special sensory function of these structures (*cf.* de Nabias, 1898) is certainly conceivable, but there is no good basis for emphasizing photoreception.

A combined light- and electron-microscope study of neurosecretory phenomena in another basommatophoran snail, *Helisoma tenue,* now in progress, has demonstrated that the structural characteristics of the follicle gland in this animal are in accord with those described herein for *Lymnaea.*

In sum, the ultrastructural attributes of the follicle gland fail to support a neurosecretory role for this organ, and the data in favor of a photoreceptive function are meager at best. The true nature of the follicle "gland" remains unknown, but a sensory function of some sort, at least in embryonic life, remains a possibility.

SUMMARY

The follicle gland of *Lymnaea auricularia* has been investigated with the light and the electron microscopes for evidence of neurosecretory function. Paraldehyde fuchsin-staining material is present in the follicle gland. At the electron-microscope level numerous microvilli and some cilia are found projecting into the lumen from the cells forming the vesicle wall. Two cell types, one epithelioid and the other neuron-like, contribute to the make-up of the follicle. The microvilli project from the apical ends of the epithelioid cells, and the cilia are attached to processes of the neuron-like cells. No elementary neurosecretory granules were encountered in the follicle gland, and the role of the organ is enigmatic, although a sensory function in early life remains a possibility.

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LITERATURE CITED

Bern, Howard A. & Irvine R. Hagadorn

1964. Neurosecretion. Chapter 6 in Bullock & Horridge: Structure and function in the nervous system of invertebrates. W. H. Freeman Co., San Francisco (in press)

Clark, Allen W.

1963. Fine structure of two invertebrate photoreceptor cells. Journ. Cell Biol. **19:** 14A (abstract)

de Nabias, B.

1898. Recherches sur le système nerveaux des Gastéropodes Pulmonés aquatiques. Cerveau des Limnées (*Limnaea stagnalis*). Soc. Sci. d'Arcachon (Stat. Zool.) : 43 - 72

Eakin, Richard M.

1963. Lines of evolution of photoreceptors. In: General physiology of cell specialization, D. Mazia and A. Tyler, *eds.*, 393 - 425, McGraw-Hill, New York

Freeman, James A. & Ben O. Spurlock

1962. A new epoxy embedment for electron microscopy. Journ. Cell Biol. **13:** 437 - 443

Lever, J.

1958. On the occurrence of a paired follicle gland in the lateral lobes of the cerebral ganglia of some Ancylidae. Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, C **61:** 235 - 242

Lever, J., H. H. Boer, R. J. Th. Duiven, J. J. Lammens & J. Wattel

1959. Some observations on follicle glands in pulmonates. Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, C **62:** 139 to 144

Lever, J. & J. Joosse

1961. On the influence of the salt content of the medium on some special neurosecretory cells in the lateral lobes of the cerebral ganglia of *Lymnaea stagnalis*. Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, C **64:** 630 - 639

Nishioka, Richard S., Irvine R. Hagadorn & Howard A. Bern

1962. Ultrastructure of the epistellar body of the octopus. Zeitschr. Zellforsch. **57:** 406 - 421

Nolte, A.

1963. Eine Cerebraldrüse bei Heliciden (Gastropoda). Licht- und elektronenoptische Untersuchungen. Gen. Comp. Endocrinol. **3:** 721 - 722 (abstract)

Pelseneer, P.

1901. Études sur des Gastéropodes Pulmonés. Mém. Acad. Roy. Belgique **54:** 1 - 76

Reynolds, Edward S.

1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. Journ. Cell Biol. **17:** 208 - 212

Röhlich, P. & L. J. Török

1963. Die Feinstruktur des Auges der Weinbergschnecke (*Helix pomatia* L.). Zeitschr. Zellforsch. **60:** 348 - 368

Sanchez, Simone & Colette Bord

1958. Origine des cellules neurosécrétrices chez *Helix aspersa* Mull. C. R. Acad. Sci. **246:** 845 - 847

Van Mol, J. - J.

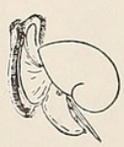
1960. Étude histologique de la glande céphalique au cours de la croissance chez *Arion rufus* Linné. Ann. Soc. Roy. Zool. Belgique **91:** 45 - 55

Watson, M. L.

1958. Staining of tissue sections for electron microscopy with heavy metals. Journ. Biophys. Biochem. Cytol. **4:** 475 - 478

Wautier, J., M. Pavans de Ceccatty, M. Richardot, B. Buisson & M. - L. Hernandez

1961. Note sur les complexes neuro-endocriniens de *Gundlachia* sp. (Mollusque Ancylidae). Bull. Mens. Soc. Linn. Lyon **30:** 79 - 87



Explanation of Plate 1

Figure 1: Lateral lobe attached to portion of cerebral ganglion. Follicle gland (fg) containing small amount of fuchsinophilic material is outlined by broken line.
ca: capsule; cg: cerebral ganglion; ll: lateral lobe. Paraldehyde fuchsin with counterstains. x870.

Figure 2: Portion of follicle gland sectioned centrally to show dense concentration of microvilli and cilia projecting into lumen. Two epithelioid cells (ec) with nuclei and three processes of neuron-like cells (nc) are shown.

Explanation of Plate 2

Figure 3: Montage of an elongate follicle gland sectioned at the periphery of the lumen (lu). Microvilli and cilia fill the lumen which is divided into two parts in this plane of section. Neuron-like cells and processes (nc) are less dense and contain irregular mitochondria. Some neuron-like cells are located external to the follicle wall and send processes through the capsule into the lumen. Epithelioid cells (ec) have small nuclei and dense cytoplasm. Connective tissue capsule (ca) separates the follicle gland from the lateral lobe.

Explanation of Plate 3

Figure 4: Section through wall of follicle gland. Neuron-like cell processes (nc) contain irregular mitochondria (m_n) and less cytoplasmic structure. The epithelioid cell processes (ec) contain many ribosomes and typical mitochondria (m_e). Soma of a neuron-like cell, with its nucleus (n_n), as indicated by the nature of its mitochondria, is located at the bottom of the figure. Small double-walled cavities (mv) in cell processes presumably represent sections through bases of microvilli. c: cilium.

Explanation of Plate 4

Figure 5: Apical part of neuron-like cell processes (nc) with three cilia (c) and basal bodies (bb) are shown. Irregular mitochondria (m_n) are present in the process. Parts of two epithelioid cells (ec) containing many ribosomes are also shown. m_e: mitochondrion of epithelioid cell; mv: microvilli; ps: polystyrene particles.