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The Fine Structure of Sensory Receptor Processes in the Auricular Epithelium of the Planarian, *Dugesia tigrina**

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Summary. The marginal epithelium of the lateral auricles of the planarian, *Dugesia tigrina*, includes a cell type with surface cilia and microvilli, a basal nucleus, and dense cytoplasm containing secretory vacuoles, Golgi elements, mitochondria and ribosomes. Through channels within the epithelial cytoplasm, cellular processes, interpreted as extensions of neurosensory receptor cells located in the subepidermis, project to the surface. The receptor processes, containing microtubules, mitochondria, vesicles and an agranular tubular reticulum, project beyond the epithelial cell surface; one or two cilia each emerge from a basal body in the apex of the projection. Close to the point of emergence to the epithelial surface, each cylindrical receptor process is surrounded by a collar-like septate junction between adjacent plasma membranes. The cilia of the projections differ from those of the epithelial cells in diameter, density of matrix and in the banding patterns of the rootlets. A few projections appear with the apex and basal body retracted below the epithelial surface. The possible function of these ciliated processes in sensory reception is discussed.

Introduction

The presence of abundant sensory receptors at the turbellarian body surface where they can respond to chemical and mechanical stimuli is rather well established. The evidence includes descriptions of their morphology, among the more detailed being those made by GELEI (1930) in rhabdocoels (see summary by HYMAN, 1951). The receptors are considered as neurosensory cells which possess a nucleated cell body in or below the epidermis, send to the surface slender processes terminating in one or more hairs or bristles, and send into the interior a nerve fiber connecting with the nervous system.

The present investigation provides information on the fine structure of the epithelium of the auricular and adjacent areas of the turbellarian, *Dugesia tigrina*, with particular reference to the structure of the cilia-bearing processes located amid the ciliated epithelial cells. These processes are interpreted as the receptor processes of neurosensory cells. Emphasis is placed on several structural features not seen by light microscopy in the receptor processes, namely, the septate junction surrounding the apical ends of the processes, their microtubules, vesicles and agranular tubular reticulum, and details of their ciliary and rootlet morphology

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distinct from those of epithelial cilia and rootlets. Structural differences between two main types of processes observed suggest that they may belong to two different sensory receptors. A preliminary note of this study appeared earlier(Mac Rae, 1967).

Material and Methods

The planarian *Dugesia tigrina* used in these experiments was maintained in culture in the laboratory in a 1:1 mixture of boiled tap water and distilled water. The animals were fed beef liver twice weekly.

The auricles and the adjacent lateral margins were removed by making razor blade cuts parallel to the long axis of the animal, and fixed immediately in cold chrome-osmium tetroxide (Dalton, 1955) or in 5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 for 1 hour. The glutaraldehyde-fixed material was post-fixed in chrome-osmium for 1 hour after a brief washing in buffer. Fixation was followed by rapid dehydration in increasing strengths of ethyl alcohol and then by orientation and flat embedding (Eakin and Westfall, 1965) in Epon (Luft, 1961). Thin sections were cut on a LKB Ultrotome III or a Porter-Blum MT-2 microtome using glass or diamond knives, and mounted on carbon-coated parlodion films supported on copper grids. Sections were double-stained on drops of 1% aqueous uranyl acetate for 30 minutes followed by 2—3 minutes with lead citrate (Watson, 1958; Reynolds, 1963) with distilled water rinse after each stain. Observations were made on the RCA-EMU 3H and the Hitachi EMU-11A. Light microscopy observations were made on thick sections after staining with a 1:1 mixture of 2% toluidine blue and 2% sodium borate.

The electron micrographs presented are all of chrome-osmium fixed material unless stated otherwise as prefixed with glutaraldehyde.

Observations

The epithelium of the planarian auricles consists of ciliated columnar cells (about 5 μ tall) with a basally located nucleus (Figs. 1, 2). Among epithelial cells there occurs cytoplasm of "insunk" cells whose nucleated cell body is in the subepithelial parenchyma. When the cells are viewed in sections perpendicular to the surface, a specialized junction consisting of septae or bridges across a uniform intercellular space is present between the plasma membranes of the apical portions of adjacent cells.

The epithelial cytoplasm appears dense and filled with mitochondria and numerous membrane-bounded vacuoles (500—2000 Å in diameter) whose contents vary from an amorphous to a densely granulated material (Figs. 2—4). The low density vacuoles appear in the vicinity of a rather extensive Golgi complex situated in the basal portion of the cell; denser and granulated vacuoles appear toward the epithelial surface and apparently empty onto the surface of the animal. Several stages in the process of change and depletion of the vacuole are shown in Fig. 4. Outside the cell, the contents appear to undergo further changes probably associated with hydration and viscosity increase.

The relationship of the basal boundary of the epithelial cell to the underlying extracellular elements is seen in Fig. 5. The basement membrane of light microscopy appears to be composed of two main components, a thin (500 Å) finely filamentous layer, the basement lamina, and a 5000 Å layer of fibrils, the basement lamellae. The fibrils are less than 100 Å thick and oriented parallel to the surface and at right angles to each other as well as obliquely. Between the basement lamina and the epithelial cell boundary is a network of electron dense material which appears at higher resolution to be attached to or continuous with

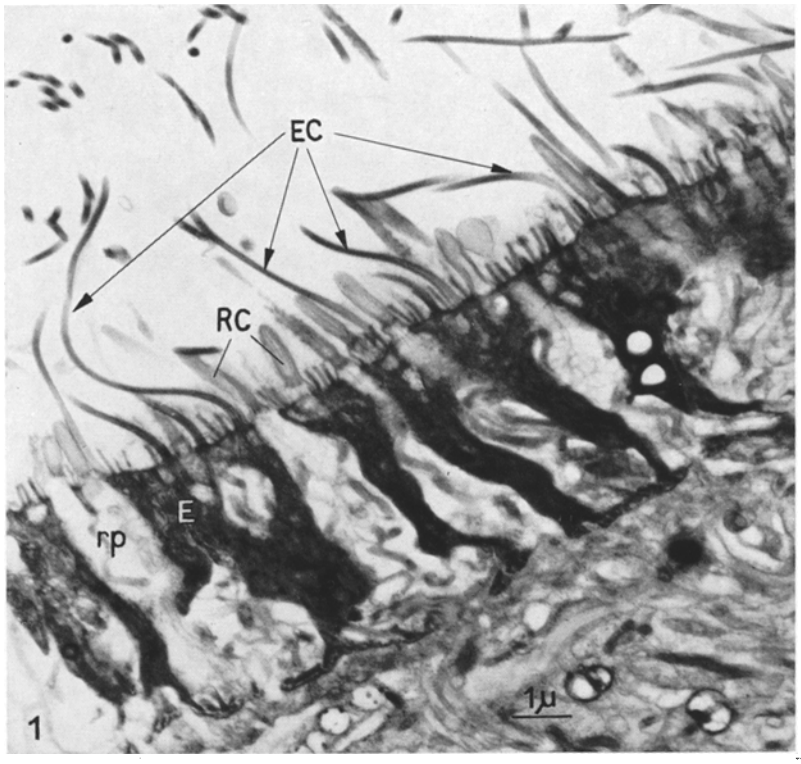


Fig. 1. Thick section through auricle, showing epithelial cell dense cytoplasm (*E*) and long cilia (*EC*) in a metachronal wave. Receptor processes (*rp*) pass through cytoplasm to the surface and end in thicker cilia (*RC*). × 8,000

the outer leaflet of the unit membrane. This network may be analogous to the globular layer in amphibian epithelium reported by WEISS and FERRIS (1954) and further described by KELLY (1966). Densities on the basal plasma membrane may be a type of hemidesmosome (Fig. 5).

Microvilli, projecting from the surface are 0.5 to 0.8 μ long, 700 to 1000 Å wide, and appear to contain longitudinal fibrillae within their matrix (Figs. 3, 4, 14).

The plasma membrane is continuous with that of the ciliary shaft containing the axonema. From the basal body located at and oriented perpendicular to the surface, a striated rootlet extends distally into the cytoplasm, and the ciliary shaft projects out apically (see EC in Fig. 3). The epithelial cilia will subsequently be discussed in greater detail in comparison with cilia of the receptor processes.

The epithelial cytoplasm is pierced by channels made by extensions or necks of secretory cells and by processes resembling nerve fibers. The extensions of secretory cells of the subepithelial parenchyma contain membrane-bounded granules (0.3—0.5 μ in diameter) filled with a finely dispersed material. The granules occur in single or several rows all surrounded by a peripheral ring of microtubules (200 Å in diameter) oriented parallel to the long axis of the neck; a cross-section is seen in Fig. 6. In longitudinal sections (Fig. 7) the microtubules

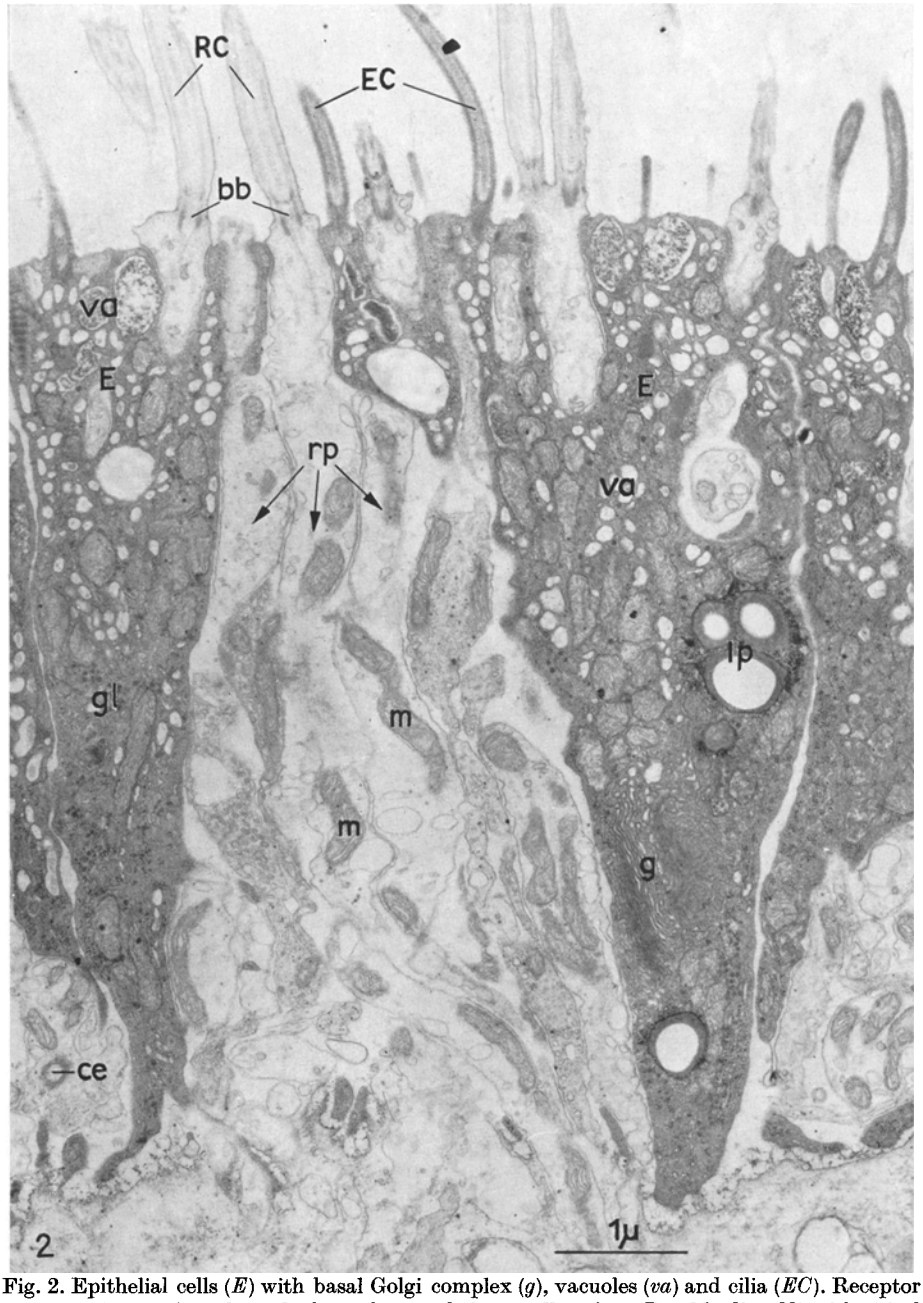


Fig. 2. Epithelial cells (*E*) with basal Golgi complex (*g*), vacuoles (*va*) and cilia (*EC*). Receptor processes (*rp*) project through channels to end above cell surface. Basal bodies (*bb*) with apical cilia (*RC*); centriole (*ce*) in process at lower left. *gl* glycogen; *lp* lipid bodies; *m* mitochondria. × 17,000

appear to make contact with the septate junction situated at the opening of the neck to the surface. Granules, at the opening of a neck, appear in the process of

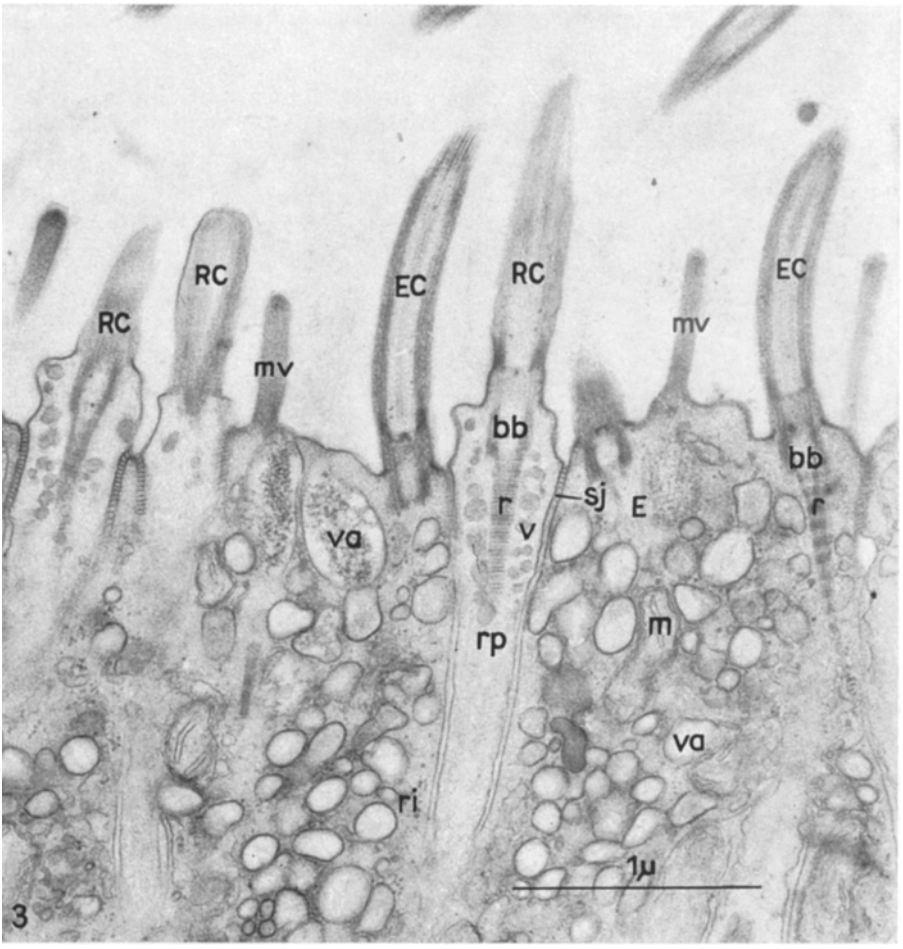


Fig. 3. Details of receptor process (*rp*); *r* rootlet; *bb* basal body; *RC* cilia; *v* vesicles. Epithelial cell (*E*) components are vacuoles (*va*), mitochondrion (*m*), microvilli (*mv*), ribosomes (*ri*) and epithelial cilia (*EC*) with rootlet (*r*) and basal body (*bb*). *sj* septate junction. × 33,000

liberation to the exterior (Fig. 7); fragments of microtubules and membranes appear to be liberated with the granules. The granules are basophilic with toluidine blue staining and similar to those already described as cyanophilic secretions in various triclads (PEDERSEN, 1959, 1963; KLUG, 1960; SKAER, 1961).

The other channels passing through the epithelial cytoplasm contain processes averaging 0.4 μ in diameter, which are characterized by a less dense cytoplasm with vesicles, mitochondria and microtubules, the latter running parallel to the long axis of the process. These processes have a morphological similarity to nerve fibers described previously in planarians (MACRAE, 1964; MORITA and BEST, 1966), and are interpreted as cytoplasmic extensions of neurosensory receptor cells whose cell bodies are located in the subepithelial parenchyma. Groups of processes usually pass together from the subepithelium into the epithelial layer but they separate and pass individually ending in ciliated projections. These can be observed in

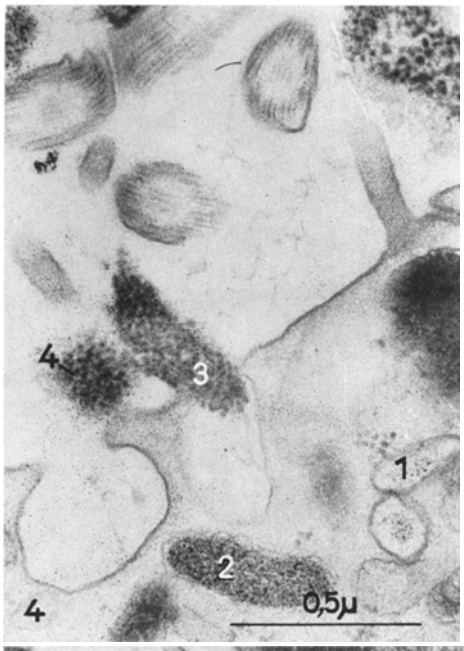
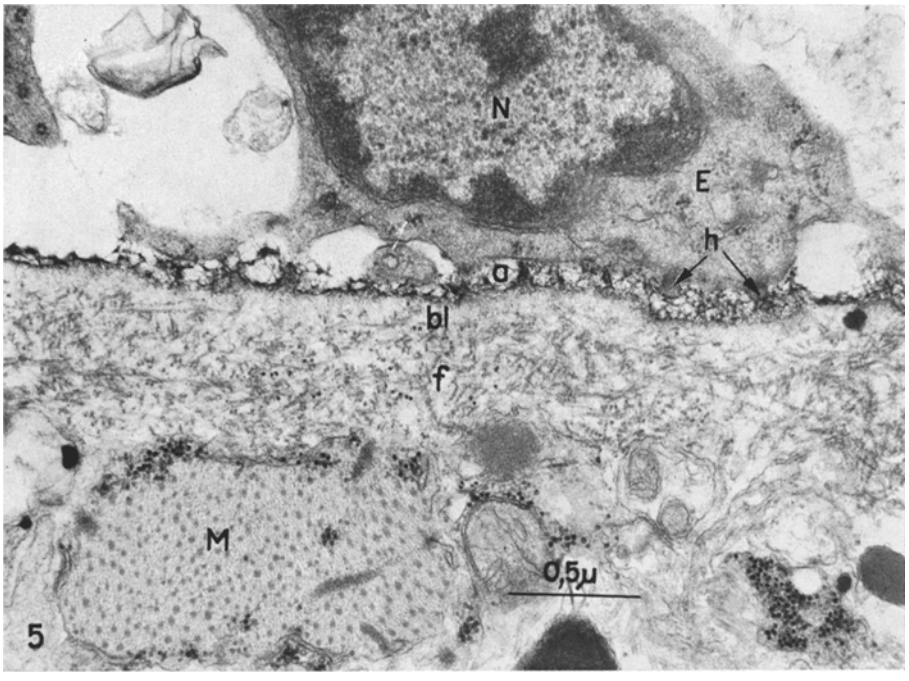


Fig. 4. Several secretory stages in the epithelial cell. *1* and *2* membrane-bounded granules in the cytoplasm, *3* granule being secreted, *4* granule having left its original investing membrane. × 50,000

Fig. 5. Epithelial cell (*E*) and underlying extracellular elements; *a* adepidermal network; *bl* thin basement lamina; *f* fibrous basement lamellae; *h* hemidesmosomes. *N* nucleus; *M* muscle cell. Glutaraldehyde with chrome-osmium post-fixation. × 33,000



sections both perpendicular and parallel to the surface (Figs. 2, 3, 11, 12). Cross-sections (Figs. 9—12) show the processes to be cylindrical. Occasionally both secretory cell extensions and receptor processes travel within the same intra-epithelial channel.

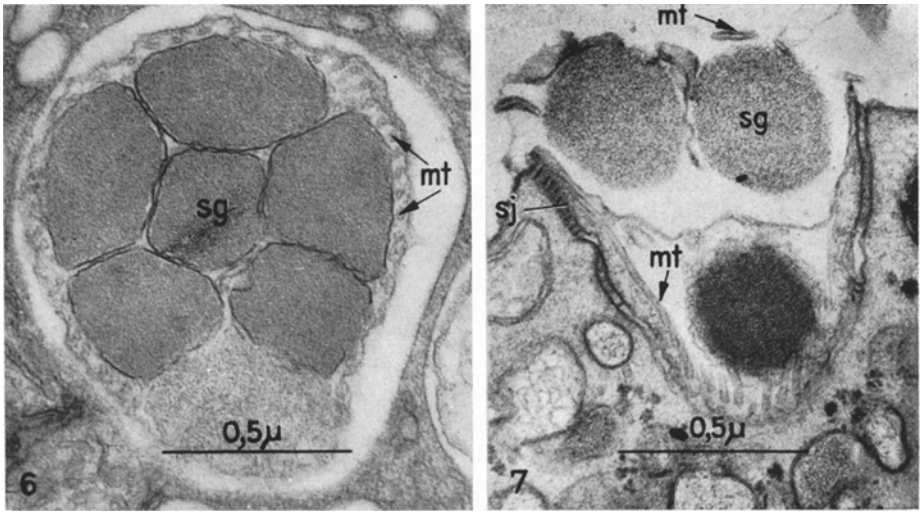


Fig. 6. Cross-section of secretory cell extension in channel within epithelial cell; *sg* secretory granule; *mt* peripheral microtubules. × 50,000

Fig. 7. Opening of cell extension to exterior; secretory granules (*sg*) and microtubules (*mt*); septate junction (*sj*) between epithelial and secretory cell. × 50,000

Each receptor process ends as a projection about 0.25 μ beyond the epithelial surface and contains one or two basal bodies (2000 Å long) oriented parallel to the long axis of the process (Figs. 1—3). In cross-section the basal body is a cylinder of 9 peripheral sets of triplet tubules or subfibrils (Figs. 9, 10). A density internal to the subfibril ring is probably the rootlet origin (Fig. 10). Nine spokes or microtubules extend radially from the central cylinder to the periphery of the process. Between each pair of spokes a vesicle may be present as seen in Fig. 10. The vesicles vary in size, density and thickness of their walls; one or more vesicles appear more prominent as seen in cross-sectional profiles (Figs. 9, 11). At high magnifications of the apical portion of the process, an agranular reticulum may be observed (Fig. 14) in addition to vesicles and microtubules. Microtubules appear hollow in cross-sections (Fig. 11). A centriolar structure similar to the basal bodies with 9 peripheral subfibril triplets (SLEIGH, 1962) is sometimes seen perpendicular to the basal body at the apex of the process; more often one centriole or a pair at right angles to each other are observed more basally in the process (Figs. 2, 12). Cross-sections taken at various levels through the receptor processes are seen in Figs. 9 and 11 with their approximate level of cut indicated in the drawing (Fig. 8).

Cross-sections of both receptor and epithelial cilia are shown in Fig. 13. They appear different in several respects. The matrix of the epithelial cilia is more dense than that of the receptor cilia. The diameters of the subfibrils of the 9 peripheral doublets are 180—200 Å for both types of cilia, but the total ciliary diameter is 2000—2200 Å for the epithelial cilia and 2600—3000 Å for the receptor cilia. The peripheral ring diameter is also different, being 1800—2200 Å for the epithelial cilia and 2000—2400 Å for the receptor cilia. The subfibril doublets appear arranged closer to their neighboring doublets in the epithelial cilia (Fig. 13).

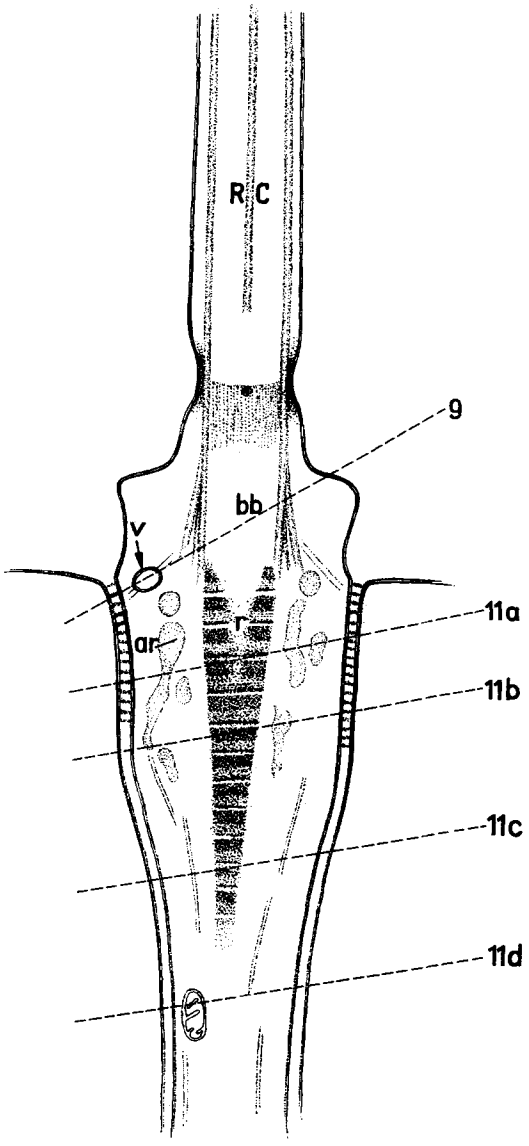
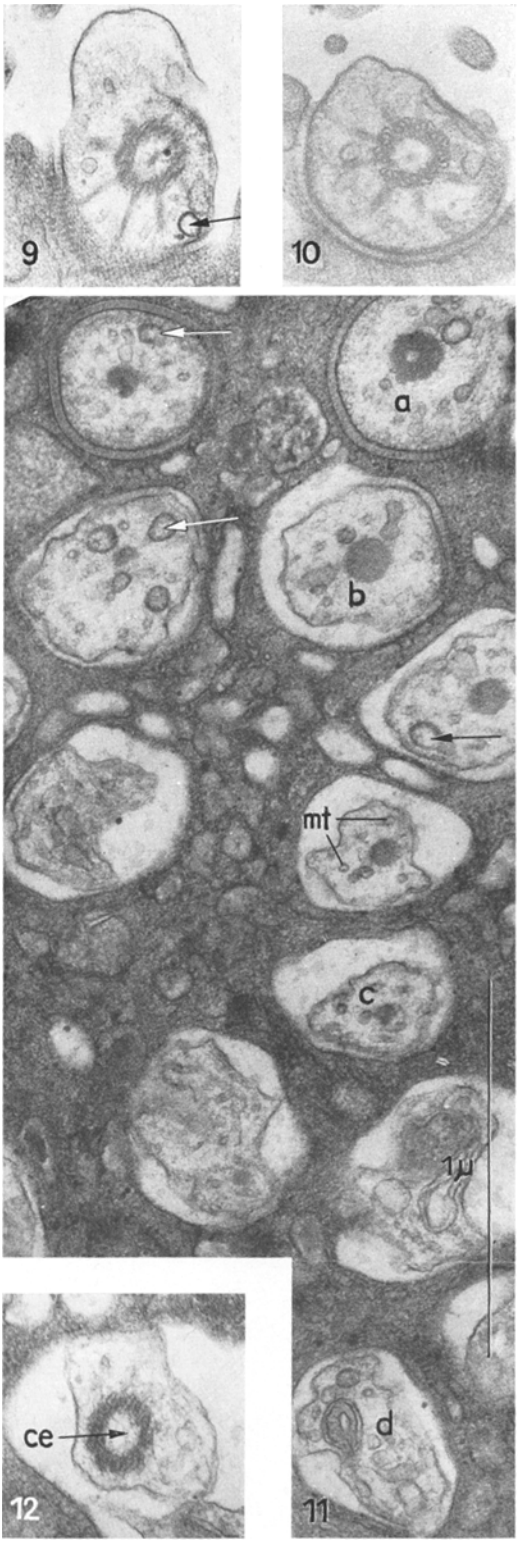


Fig. 8. Drawing through long axis of a receptor process; *bb* basal body; *r* rootlet; *RC* cilium; *v* thick walled vesicle; *ar* agranular reticulum. The transverse dotted lines through various levels of this structure show approximate planes of section of processes in Figs. 9 and 11

Some evidence suggests that the epithelial cilia may move independently of the receptor cilia. In sections made parallel to the surface and near the cilia tips, the denser epithelial cilia are in longitudinal or oblique sections, while in the same field of observation the receptor cilia are in cross-section. In Fig. 3, several receptor cilia (RC) with their basal bodies and epithelial cilia (EC) with their basal bodies are observed in longitudinal section. Note that the basal bodies,



Figs. 9 and 10 are oblique sections through basal body, radial spokes and one rim of septate junction. × 50,000

Fig. 11. An oblique section of auricle showing sections of receptor processes at several levels corresponding to dotted lines in drawing. Thick walled vesicles at arrows. *mt* microtubules. × 50,000

Fig. 12. Section through process with centriole (*ce*). × 50,000

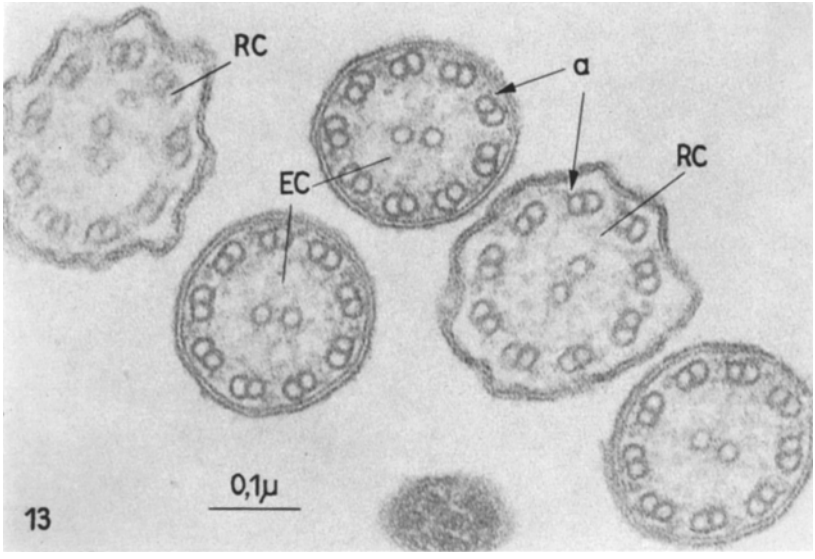


Fig. 13. Cross-sections through two ciliary types. *RC* receptor cilia; *EC* epithelial cilia. Sections viewed from apex toward base; counterclockwise arms (*a*). Glutaraldehyde fixation with chrome-osmium post-fixation. × 120,000

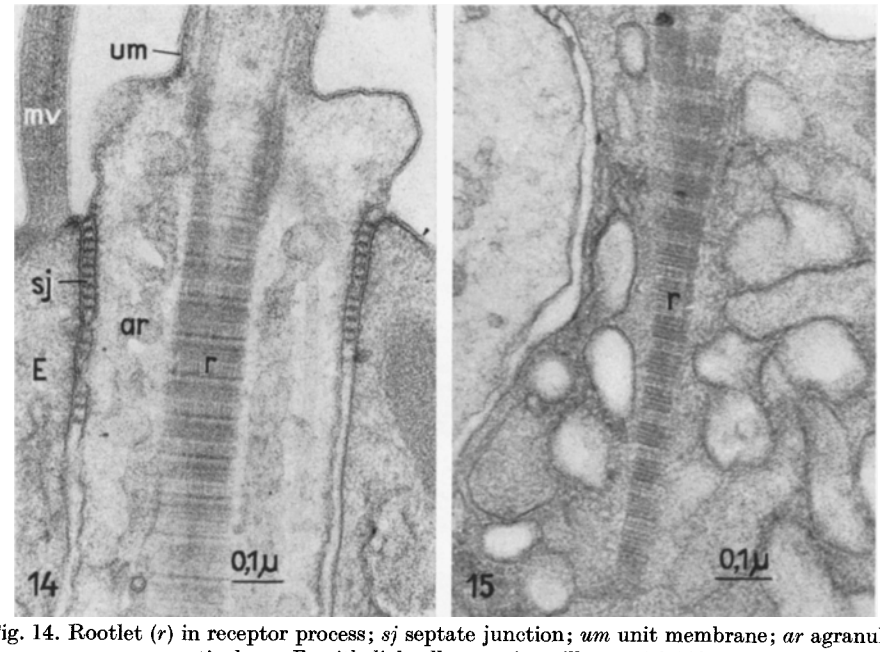


Fig. 14. Rootlet (*r*) in receptor process; *sj* septate junction; *um* unit membrane; *ar* agranular reticulum; *E* epithelial cell; *mv* microvillus. × 70,000

Fig. 15. Rootlet (*r*) in epithelial cell. × 70,000

rootlets and emerging shafts of the epithelial cilia differ in orientation from those of the receptor cilia by an angle of about 20°. Further indication of the different

orientation between the two types of cilia is presented in Figs. 1 and 2 as well. Suggestion of a metachronal wave (SATIR, 1963) among the denser epithelial cilia (EC) is seen in Fig. 1.

The presence of a side arm from one subfibril of each doublet is observed in cross-sections of both ciliary types. However, only one arm from the outer aspect of subfibril A of each doublet appears to be directed toward subfibril B of the adjacent doublet (at *a* in Fig. 13). The inner arm appears to be lacking. The arm of each doublet is directed counter-clockwise when the cilia are viewed from the apex in toward the base, or would be directed clockwise when viewed from base toward apex as in the more conventional practice (SLEIGH, 1962).

Another difference between epithelial and receptor cilia is the structural stability of the membrane. After chrome-osmium the membrane of the receptor cilia displays great variation in terms of surface outpocketings, ruffling and fragmentation with vesicular formation. Occasionally vesicles appear within the ciliary shaft between the peripheral subfibril ring and the ciliary membrane.

The rootlets extending distally from the basal bodies of each of the two types of cilia are different in their observable banding patterns. The rootlet in the receptor process (Fig. 14) includes a dark thin cross-striation followed by a clear band (about 160 Å), then a fairly uniform band of 280 Å followed by a lighter uniform band of 280 Å; the pattern repeats at approximately 680—700 Å. In the epithelial rootlet (Fig. 15), the striations include a dark band (about 360 Å thick) composed of 5 equally spaced cross-striations, then a lighter band (about 280 Å thick) including 2 faint cross-striations; the total pattern repeats itself in 640—680 Å.

The trilaminar membrane of each receptor process continues as the ciliary membrane and the inner component of the membrane appears denser (Fig. 16). Around each receptor process just before it projects beyond the epithelial surface is a collar (2800 Å deep) formed by adjacent plasma membranes. In sections parallel to the long axis of the fiber, the collar is observed as formed by the trilaminar membranes of the process and the adjacent epithelial cell; these membranes are separated by a uniform space of about 200 Å traversed by septae (80—100 Å thick) occurring at about 100 Å intervals (Figs. 3, 16). The collar appears around the total circumference of the cylindrical process, as seen in cross-sections made at upper levels of the rootlets (see Fig. 11). These junctions have the same basic structure as those between the apical ends of adjacent epithelial cells and between the terminal ends of secretory cell extensions and adjacent epithelial cell membranes (Fig. 7).

The septate junctions have been observed under high magnification in several planes of section. Since the process is cylindrical, it offers three dimensions from which to infer the plane of section and the possible morphology of the junction. It can be sectioned in a mid-sagittal or longitudinal plane with respect to the axis of the cylindrical process. This section provides evidence that the "ladder" structures formed by the septae across the intercellular space project from the outer component of the adjacent unit membranes (Fig. 16). When a section is made sagittal but not through the mid-line of the cylinder, the septae are so sectioned as to appear as shelves 100 Å apart and having a slight ridging at about 160 Å (Fig. 17). A section made tangential to the cylinder displays a hexagonal pattern (Fig. 18) not observed when the section is further into the cylinder. When

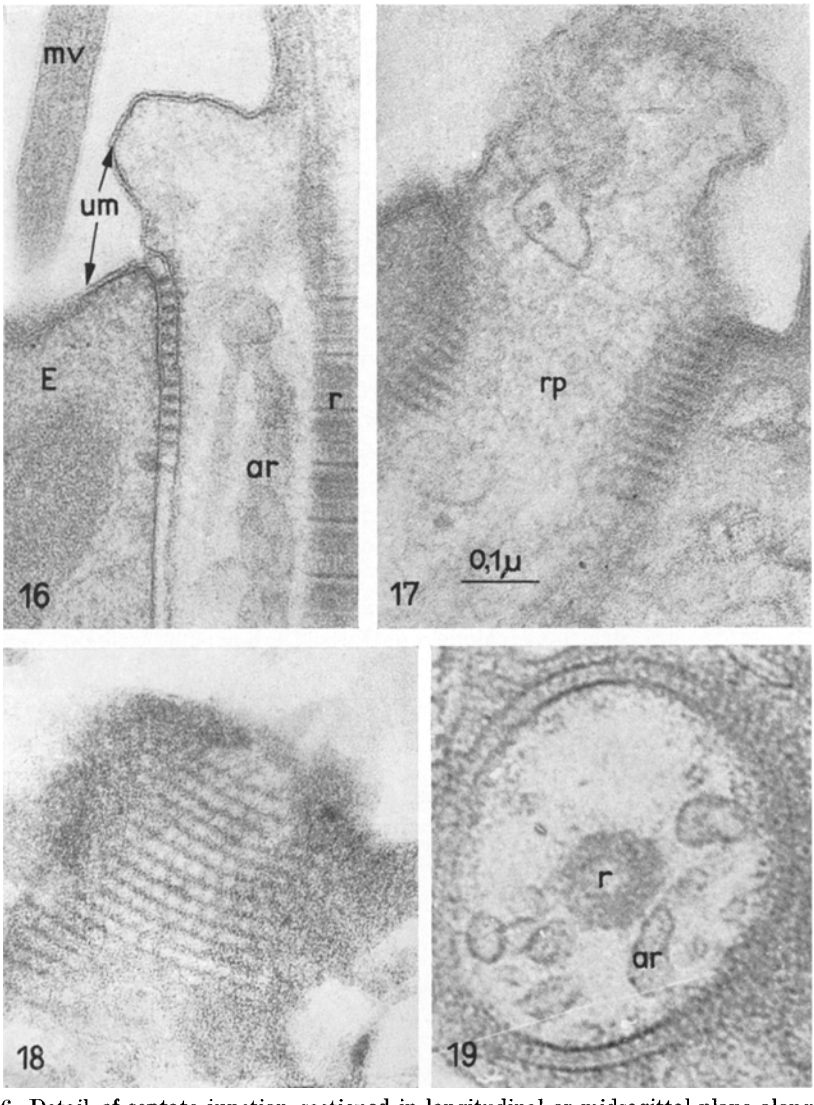


Fig. 16. Detail of septate junction sectioned in longitudinal or midsagittal plane along long axis of process; *r* portion of rootlet; *ar* agranular reticulum; *um* unit membrane around receptor process and at surface of epithelial cell (*E*) continues into septate junction. × 100,000

Fig. 17. The cylinder of receptor process (*rp*) is sectioned in sagittal plane which does not pass through the mid-line. The septae appear to possess shallow ridges and form shelves across the intercellular space. × 100,000

Fig. 18. A tangential section of septate junction edge showing a hexagonal pattern. × 100,000

Fig. 19. A transverse section through receptor process and septate junction. Note a periodicity with a shorter interval than that of the hexagonal pattern; *r* rootlet; *ar* agranular tubular reticulum. × 100,000

the cylinder is cut in a transverse plane, the intercellular space between the two unit membranes may appear homogeneous, or it may have a faint striation. The homogeneous appearance is interpreted as due to a section which is cut between

two adjacent septae. The striated appearance is due to a section through a septa, the troughs and crests of the ridges so cut as to appear as striations (Fig. 19).

Occasionally there can be observed structures with the apical portion of the receptor process including the basal body and a portion of the cilium retracted distally into the process (Fig. 20). These structures are not observed very frequently but they are found scattered among the non-retracted processes. At the level of the basal body the diameter of the retracted form is about 1 μ while the diameter of the non-retracted form is 0.3—0.4 μ. Vesicles, microtubules and mitochondria are similar in both forms. Although not all surface areas have been fully examined, both types occur in the auricular epithelium and at the ventral lateral edges of the head.

The cell body from which the receptor processes originate, presumably several similar processes from one cell, is in the deep subepithelial parenchyma. The description of this cell will be published at a later time.

Discussion

Ciliated end-organs have been described in a variety of species for several sensory modalities including the vestibular and lateral line organs (Wersäll, 1956; Flock and Duvall, 1965), the auditory organs of vertebrates (Wersäll, Flock und Lundquist, 1965) and of invertebrates (Gray, 1960), the olfactory epithelium of vertebrates (De Lorenzo, 1957; Reese, 1965; Frisch, 1965) and possible chemoreceptors in insects (Slifer and Sekhon, 1961). A cilium is also associated with the development of the outer segment of retinal rods in the vertebrates (De Robertis, 1956) and with photoreceptors in one of the two main evolutionary lines of invertebrates (Eakin, 1965a).

The location of the ciliated processes described in this investigation suggest

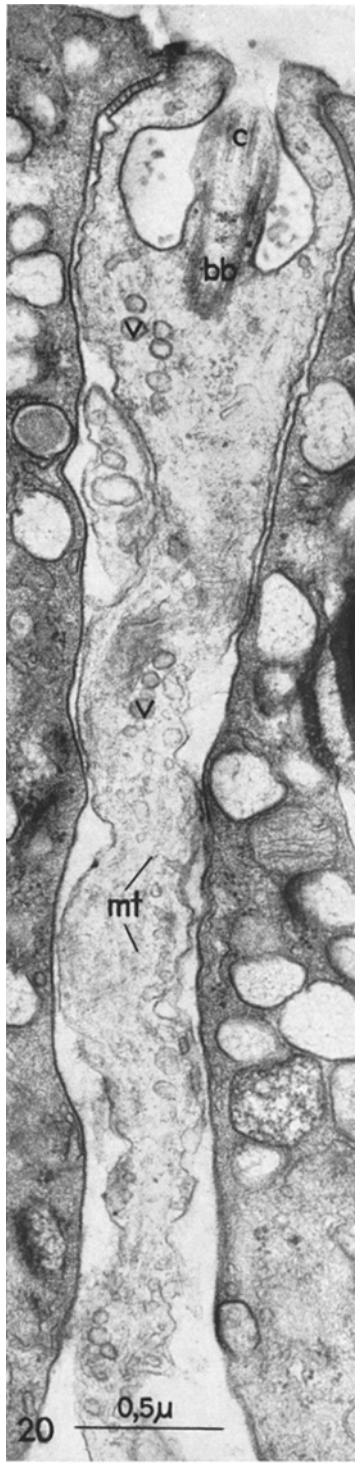


Fig. 20. Longitudinal section through a process with apical end and cilium (*c*) retracted. *bb* basal body; *v* vesicles; *mt* microtubules. × 40,000

that they may be the sensory receptor processes implicated as chemoreceptors by the work of Koehler (1932). His experiments, well summarized by Fraenkel and Gunn (1961), showed that when food is placed in a dish with planarians, the first sign of chemical excitation is a lengthening of the anterior end including the auricles. The crawling toward the food at first is undirectional and with swinging of the head from side to side; when the animal gets within 8 cms of the food, it goes straight to it. If the sides of the head including the auricles are cut off, the food is either not found at all or found after prolonged random movements. Removal of one auricle caused the animals to make repeated turns to the opposite side when tested with food. The interpretation to these behavioral responses is that the principal receptors are located in two concentrated and symmetrically placed groups of structures in the head, and the receptors plus a coordination system enable the planarians to detect and react to chemo-stimulation.

Morphologically the receptor processes resemble vertebrate olfactory endings which are a type of chemoreceptor. Olfactory receptors in the fish (Trujillo-Cenóz, 1961; Porter and Bonneville, 1964), in the frog (Reese, 1965) and the mammal (De Lorenzo, 1957; Frisch, 1965) have similar structures to the process endings described in the planarian. Differences do exist in more cilia per vertebrate dendritic ending or olfactory rod, and the location of the cell body of the olfactory nerve within the epithelium. The nucleated cell body of the planarian receptor is located in the subepithelial parenchyma and may be part of the cell mass of the brain or cerebral ganglion. However, morphological similarity is not sufficient to ascribe a similar role to these processes.

Inasmuch as contact receptors are also numerous in the auricular areas, one must consider the possibility that these processes may respond to mechanical stimuli. Among the processes observed, there may be more than one type, varying in some as yet unobserved characteristics; the detection of chemicals, water currents and contact stimuli may be performed by some structural variants of the basic form of receptor process described here. However, the retracted form of receptor process found among the epithelial cells may be a form of tactile or contact receptor as suggested by its deformability. Drawings of Gelei (1930) (see Hyman, 1951) of rheoreceptors in *Mesostoma* show structures with a broadening at the basal body level, which bear a similarity to the bulbous retracted endings shown in Fig. 20.

In what manner these sensory receptor processes and their component organelles react to the chemical or mechanical stimuli is not known and can only be speculated on. Electrical responses of olfactory epithelium to chemical stimuli have indicated that the cilia may be the point at which stimulation is initiated by contact with the chemical substances (Ottoson, 1963). A difference between the membrane of the receptor cilia and that of the epithelial cilia is suggested by the fragility of the receptor ciliary membranes. This fragility, observed particularly after chrome-osmium, is probably a fixation artefact. Nevertheless, the same fixation and preparatory procedures leave intact the epithelial ciliary membranes within the same field of observation. Fragility and vesicular formation of photoreceptor membranes have been observed after osmium fixation under certain experimental conditions by Eakin (1965b) and Röhlich (1966).

Furthermore, differences in density of the matrix, in the diameter of the axoneme, and in rootlet banding are apparent between receptor and epithelial cilia, suggesting that their fundamental structures probably reflect different functional capacities. That they act independently seems very likely from the observations on their different pattern of orientation (Fig. 3).

It seems not unlikely that chemical or mechanical contact with receptor ciliary membranes may produce a response which is transduced into nerve impulses passing to the neuron and central nervous system to effect total body motor response. Various vesicles and the agranular tubular reticulum at the level of the basal bodies may play a significant role in this transduction. One may speculate that ion communication may occur into adjacent ciliated epithelial cells to influence local response as in the rate of epithelial ciliary beating and/or glandular secretions. There are cases, as discussed by FRAENKEL and GUNN (1961), in which the initial excitation produced by molecules arriving by diffusion is increased by means of a current produced by the animal itself. A co-ordination of activity among epithelial cells in response to stimuli received may be achieved by a free flow of ions due to low resistance coupling such as has been demonstrated to occur between individual cells of the salivary gland of *Drosophila* (LOEWENSTEIN and KANNO, 1964). The location of the low resistance pathway is not known exactly but the septate junction has been implicated as a likely area for such communication.

The structure of the septate junction around the processes appears similar to those described in invertebrates by WOOD (1959), LOCKE (1965) and others, although the measurements vary somewhat. However, one main difference in interpretation is that LOCKE considered the hexagonal pattern of septate junction sectioned tangentially to extend across the intercellular space. The micrographs studied in this investigation suggest that the hexagonal pattern in these forms may be restricted to the outer plasma membranes and not continuous into the space. In sections, perpendicular to the septae and across the intercellular space, as well as in sagittal sections not through the mid-line, there was no clear indication that the septae were other than shelves across the space.

Elucidation of the functional role of these processes must await physiological data on stimulation and response at individual specific processes.

References

DALTON, A. J.: A chrome-osmium fixative for electron microscopy. Anat. Rec. **121**, 281 (1955).

DE LORENZO, A. J.: Electron microscopic observations of the olfactory mucosa and olfactory nerves. J. biophys. biochem. Cytol. **3**, 839—850 (1957).

DE ROBERTIS, E.: Morphogenesis of the retinal rods. An electron microscope study. J. biophys. biochem. Cytol. **2**, No. 4, Suppl., 209—218 (1956).

EAKIN, R. M.: Evolution of photoreceptors. Cold Spr. Harb. Symp. quant. Biol. **30**, 363—370 (1965a).

— Differentiation of rods and cones in total darkness. J. Cell Biol. **25**, 162—165 (1965b).

—, and J. A. WESTFALL: Dissection and oriented embedding of small specimens for ultramicrotomy. Stain Technol. **40**, 13—14 (1965).

FLOCK, Å., and A. J. DUVALL: The ultrastructure of the kinocilium of the sensory cells in the inner ear and lateral line organs. J. Cell Biol. **25**, 1—8 (1965).

FRAENKEL, G. S., and D. L. GUNN: The orientation of animals. New York: Dover 1961.

FRISCH, D.: Ultrastructure of mouse olfactory mucosa. Anat. Rec. **151**, 351 (1965).

Gelei, J. v.: Echte freie Nervenendigungen. Z. Morphol. Ökol. Tiere **18**, 786—798 (1930).
Gray, E. G.: The fine structure of the insect ear. Phil. Trans. B. **243**, 75—94 (1960).
Hyman, L. H.: The invertebrates, vol. II. New York: McGraw-Hill Book Co. 1951.
Kelly, D. E.: Fine structure of desmosomes, hemidesmosomes, and an adepidermal globular layer in developing newt epidermis. J. Cell Biol. **28**, 51—72 (1966).
Klug, H.: Über die funktionelle Bedeutung der Feinstrukturen der exokrinen Drüsenzellen (Untersuchungen an *Euplanaria*). Z. Zellforsch. **51**, 617—632 (1960).
Koehler, O.: Beiträge zur Sinnesphysiologie der Süßwasserplanarien. Z. vergl. Physiol. **16**, 606—756 (1932).
Locke, M.: The structure of septate desmosomes. J. Cell Biol. **25**, 166—169 (1965).
Loewenstein, W. R., and Y. Kanno: Studies on an epithelial (gland) cell junction. J. Cell Biol. **22**, 565—586 (1964).
Luft, J. H.: Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. **9**, 409—414 (1961).
MacRae, E. K.: Observations on the fine structure of photoreceptor cells in the planarian, *Dugesia tigrina*. J. Ultrastruct. Res. **10**, 334—349 (1964).
— Fine structure of sensory nerve endings within planarian auricular epithelium. Anat. Rec. **157**, 282 (1967).
Morita, M., and J. B. Best: Electron microscopic studies of planaria. III. Some observations on the fine structure of planarian nervous tissue. J. exp. Zool. **161**, 391—411 (1966).
Ottoson, D.: Some aspects of the function of the olfactory system. Pharmacol. Rev. **15**, 1—42 (1963).
Pedersen, K. J.: Some features of the fine structure and histochemistry of planarian subepidermal gland cells. Z. Zellforsch. **50**, 121—142 (1959).
— Slime-secreting cells of planarians. Ann. N. Y. Acad. Sci. **106**, 424—442 (1963).
Porter, K. R., and M. A. Bonneville: An introduction to the fine structure of cells and tissues. Philadelphia: Lea & Febiger 1964.
Reese, T. S.: Olfactory cilia in the frog. J. Cell Biol. **25**, 209—230 (1965).
Reynolds, E. S.: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **7**, 208—212 (1963).
Röhlich, P.: Sensitivity of regenerating and degenerating planarian photoreceptors to osmium fixation. Z. Zellforsch. **73**, 165—173 (1966).
Satir, P.: Studies on cilia. The fixation of the metachronal wave. J. Cell Biol. **18**, 345—365 (1963).
Skaer, R. J.: Some aspects of the cytology of *Polycelis nigra*. Quart. J. micr. Sci. **102**, 295—317 (1961).
Sleigh, M. A.: The biology of cilia and flagella. London: Pergamon Press 1962.
Slifer, E. H., and S. S. Sekhon: Fine structure of the sense organs on the antennal flagellum on the honey bee, *Apis mellifera Linnaeus*. J. Morph. **109**, 351—382 (1961).
Trujillo-Cenóz, O.: Electron microscope observations on chemo- and mechanoreceptor cells of fish. Z. Zellforsch. **54**, 654—676 (1961).
Watson, M. L.: Staining of tissue sections for electron microscopy with heavy metals. J. biophys. biochem. Cytol. **4**, 476—478 (1958).
Weiss, P., and W. Ferris: Electron micrograms of larval amphibian epidermis. Exp. Cell Res. **6**, 546—549 (1954).
Wersäll, J.: Studies on the structure and innervation of the sensory epithelium of the cristae ampullares in the guinea pig. Acta oto-laryng. (Stockh.), Suppl. **126**, 1—85 (1956).
— Å. Flock, and P. Lundquist: Structural basis for directional sensitivity in cochlear and vestibular sensory receptors. Cold Spr. Harb. Symp. quant. Biol. **30**, 115—132 (1965).
Wood, R. L.: Intercellular attachments in the epithelium of Hydra as revealed by electron microscopy. J. biophys. biochem. Cytol. **6**, 343—352 (1959).

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