FINE STRUCTURES OF TURTLE OLFACTORY RECEPTOR CELLS

Tsuneo HATANAKA

(Department of Biology, Faculty of Education, Chiba University)

ABSTRACT

The fine structure of the olfactory mucosa and respiratory epithelium in the Reeve's turtle were observed with scanning and transmission electron microscope. The surface of the olfactory mucosa was covered with long olfactory cilia densely. Whereas, at the respiratory mucosa, tufted short cilia were observed. Olfactory receptor cells of olfactory mucosa bore several cilia from its dendrite terminal swelling and ciliated epithelial cells of respiratory mucosa also have both short cilia and microvilli between cilia. In both mucosa, cells which contained many secretory granules were observed (supporting cells in olfactory mucosa and goblet cells in respiratory mucosa). Functional differences between cilia of olfactory receptor cells, those of ciliated epithelial cells and microvilli of vomeronasal receptor were discussed.

INTRODUCTION

Generally, vertebrates have two olfactory systems, olfactory mucosa and vomeronasal organ. The olfactory mucosa is located in nasal cavity, but the vomeronasal organ is generally isolated from it and connected with nasal or oral cavity by fine duct in snakes, lizards and mammals. But in aqueous turtles, such as Reeve's turtle or red eared turtle, the olfactory nerve distributes on dorsal part of the nasal cabity, and the vomernasal nerve innervates to ventral region of it. So two types of olfactory receptor mucosa segregate on the nasal cavity.

As yet, vomernasal receptors have been reported to be microvillous type which posses microvilli only at the dendrite terminal without exception (Adams & Wiekamp, 1984; Altner & Muller, 1968; Graziadei & Tucker, 1970; Kratzing, 1975; Takami and Hiroswa, 1990; Vaccarezza et al., 1981; Wang & Halpern, 1980). Whereas, olfactory receptor cells are generally ciliated type, but microvillous type olfactory receptors were founded in fishes, newts and rats (Bannister,1965; Kratzing, 1975; Matsuzaki et al., 1982; Okano et al.,1967; Reese, 1965; Rowley et al., 1989; Usukura and Shibuya ,1974). Vomeronasal receptors in aqueous turtles, such as Reeve's turtle and red eared turtle

also have microvilli (Hatanaka et al., 1981; Hatanaka and Hanada, 1986). Morphological differences berween these two species of olfactory receptor cells would reflected to the differences of their functions. It is required to ascertain the receptor type of olfactory receptors which occupies the same nasal cavity with vomeronasal receptors.

In aqueous turtles, the number of vomeronasal receptor cells are little larger than that of olfactory ones. So two types of olfactory system are well developed and seems to be fully functional. To consider the role of two olfactory system, it must be studied the morphology of two types of receptor cells. Fine struture of the vomeronasal receptors in Reeve's turtle and red eared turtles were reported previously. In this paper, fine structure of the olfactory mucosa and respiratory mucosa which separated between the vomeronasal mucosa and olfactory one were observed with scanning and transmission electron microscope.

MATERIALS AND METHODS

Reeve's turtle (*Geoclemys reevesii*) used were 180 to 880g in body weight. The animal was lightly anesthetized with ethyl urethane (0.2g／100g b.w.) and fixed by perfusion of the fixative into the artery. Fixative was prepared with 2.5% glutaraldehyde and 2% formaldehyde in 0.2 M cacodylate buffer at pH. 7.4. Then the whole nasal cavity was isolated with cartilage surrounding the organ.

For the preparations observed with light microscope, entire cartilaginous capsule was re-fixed with same fixative, and then post-fixed with buffered 2% osmium tetraoxide. After fixation, the capsule was dehydrated and embedded in Epon. Sections 2 to 3 μm thick were cut transversally and stained with fuchsine methylenblue.

For the scanning electron microscope, The isolated tissue wsa divided in two horizontally, and washed gently with physiological solution or buffered solution for 30 minutes to an hour to remove the mucus. And then re-fixation with same fixative and post-fixation with osmium tetraoxide were carried out. After fixation, tissue was dehydrate, dried and coated with evapolated gold.

For the transmission electron microscope, the isolated tissue was dissectede into suitable size and tissue pieces were immersed in same fixative and post-fixed with osmium tetraoxide. After fixation, the tissue was dehydrated and embedded in Epon. Thin sections were stained with lead citrate and uranyle acetate.

RESULTS

1. Light microscopic observation

Transverse section of the nasal cavity through the central portion shows that the olfactory mucosa occupied the dorsal region and the vomeronasal mucosa was located in the ventral region. The respiratory mucosa was situated on both lateral side of the middle part separating two kinds of receptor mucosa. These three types of mucosa could be easily distinguished by microscope from their surface structures. In the olfactory mucosa,

numerous long olfactory cilia horizontally over the surface were observed. While in the respiratory mucosa, short and straight cilia were seen. However, such ciliary structure was never visible in the vomeronasal-nasal region (Hatanaka et al., 1981). In these three types of mucosa, long cylinderic cells which contained good stained secretory granules near the surface were observed. These cells were supporting cells in receptor mucosa and goblet cells in respiratory mucosa. Ratio of the length of three mucosa in a transverse section through the central line of the cavity was following ; vomeronasal : respiratory : olfactory mucosa $=1:1.07:1.15$.

Olfactory mucosa was composed by three types of cell layer, supporting cells, receptor cells and basal cells. Supporting cells lined the upper region, while numerous receptor cells with round somata were under the supporting cell layer. Receptor cell dendrites extended between supporting cells and they bore olfactory cilia from their terminal.

In the respiratory region, ciliated epithelial cells whose diameter of the cell surface was enlarged had numerous thick cilia. And goblet cells including many secretory granules were observed.

2. Scanning electron microscopic observation

Using the scanning electron microscope, the differences of three types of mucosa could be recognized easily from the surface structure. At the surface of the olfactory mucosa, entangled long olfactory cilia which were 0.26 - 0.33 μm in thickness and more than 20 μm in length covered densely over the surface (Fig. 1-A). In the fractured preparation, an isolated dendrite terminal (olfactory vesicle) of olfactory receptor cell was seen. On the terminal, several thick cilia were borne and many small microvilli with about 0.1 μm in thickness and 2.1 μm in length surrounded them closely (Fig. 1-B). Accordingly, the surface of olfactory mucosa was covered with cilia and mirovilli, and free surface of receptor cells and supporting cells was seldom seen.

In the respiratory mucosa, tufted cilia which were 0.3 -0.5 μm thick and 2.5 - 5.5 μm long were observed (Fig. 1-B). At the small part where the density of ciliated cell was low, the surface of the goblet cell could be seen, and sometimes scores of microvilli which were 0.13 - 0.18 μm thick and 0.3 μm long were observed there.

3. Transmission electron microscopic observation

In the olfactory mucosa, terminal of receptor dendrite was swollen and protruded beyond the mucosal surface making olfactory vesicle or olfactory knob, from which olfactory cilia were born (Fig. 2-A). Dendrite terminal was 1.5 - 2 μm in diameter and bore 5 - 10 olfactory cilia with 0.2 - 0.3 μm in thickness. Above the layer of microvilli (about 2 μm in thickness), olfactory cilia bend and run along the mucosal surface, so cross sections of cilia were observed densely. Olfactory cilia were born at the lateral side of olfactory vesicle radially, and also microvilli were seen at more distal region of olfactory vesicle. Some basal bodies of cilia were observed in

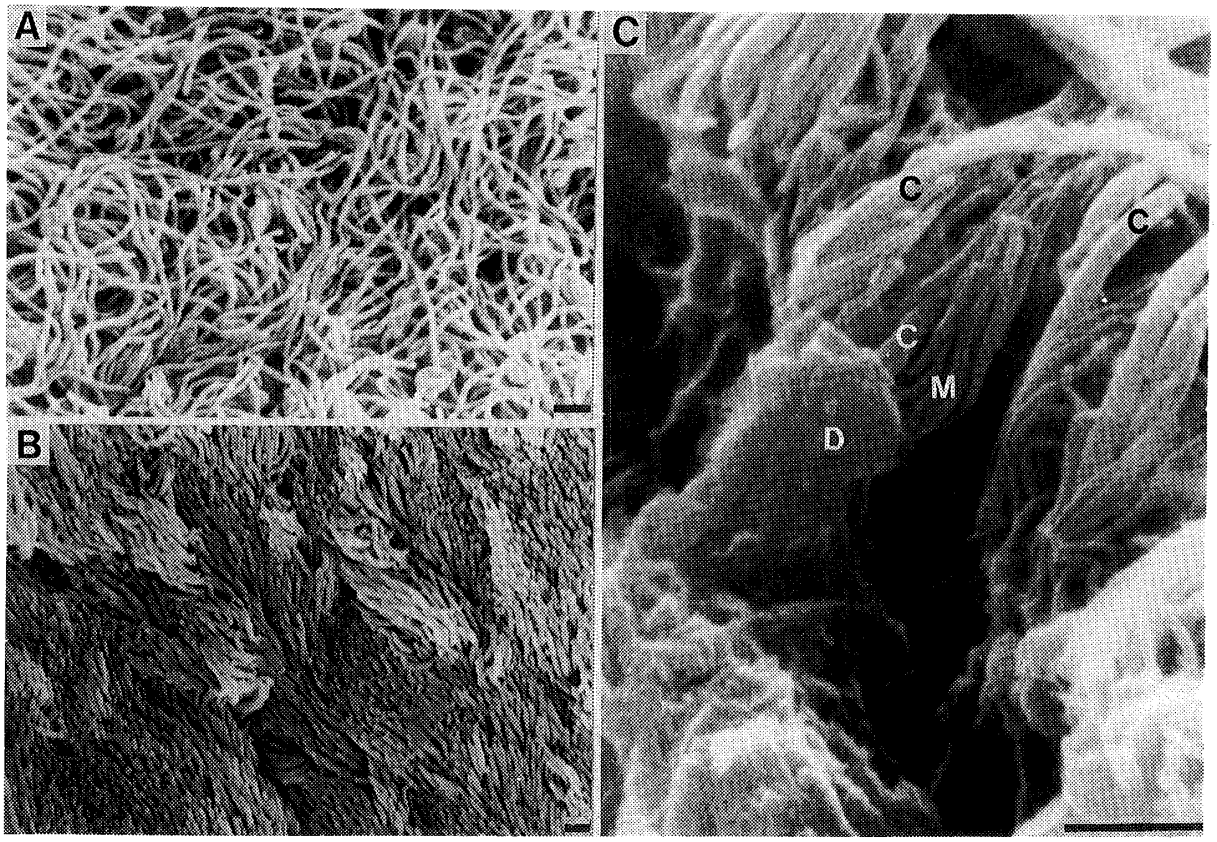

Fig. 1 Scanning electron micrograph of turtle nasal cavity.
A: Surface structure of the olfactory region. Entangled long olfactory cilia covers densely over the surface. Scale; 1 μm.
B: Surface structure of the respiratory region. Straight and short cilia of each ciliated epithelial cell are tufted. Scale; 1 μm.
C: Fractured preparation of olfactory mucosa. An isolated receptor terminal (D) with thick cilia (C) and thin microvilli (M) are visible. Scale; 1 μm.

the olfactory vesicle. Through the dendrite, neurotubles and numerous slender mitochondria were arranged longitudinally (Fig. 2-A). Nuclei of receptor cells were stained in lower than those of supporting cells, and they showed irregular shape (Fig. 2-B). In the perikaryon, a thin layer of cytoplasm surrounded the mucleus and rough surface endoplasmic reticulum were observed. Olfactory receptor cells had higher electron density than supporting cells, so they could easily distinguished. Supporting cells were cylindrical in shape with 5 - 6 μm in diameter and several scores micron meter in length. They had many microvilli 2 - 3 μm in length and less than 0.2 μm in thickness. In the distal region and above the nuclei, numerous secretory granules of 1.1 - 1.8 μm in diameter were packed, and in the middle part, large mitochondria surrounded rough surface endoplasmic reticulum and Golgi apparatus (Fig. 2-D). In the secretory granule, high density substances were packed in about 1／2 - 1／3 of volume. Receptor dendrite

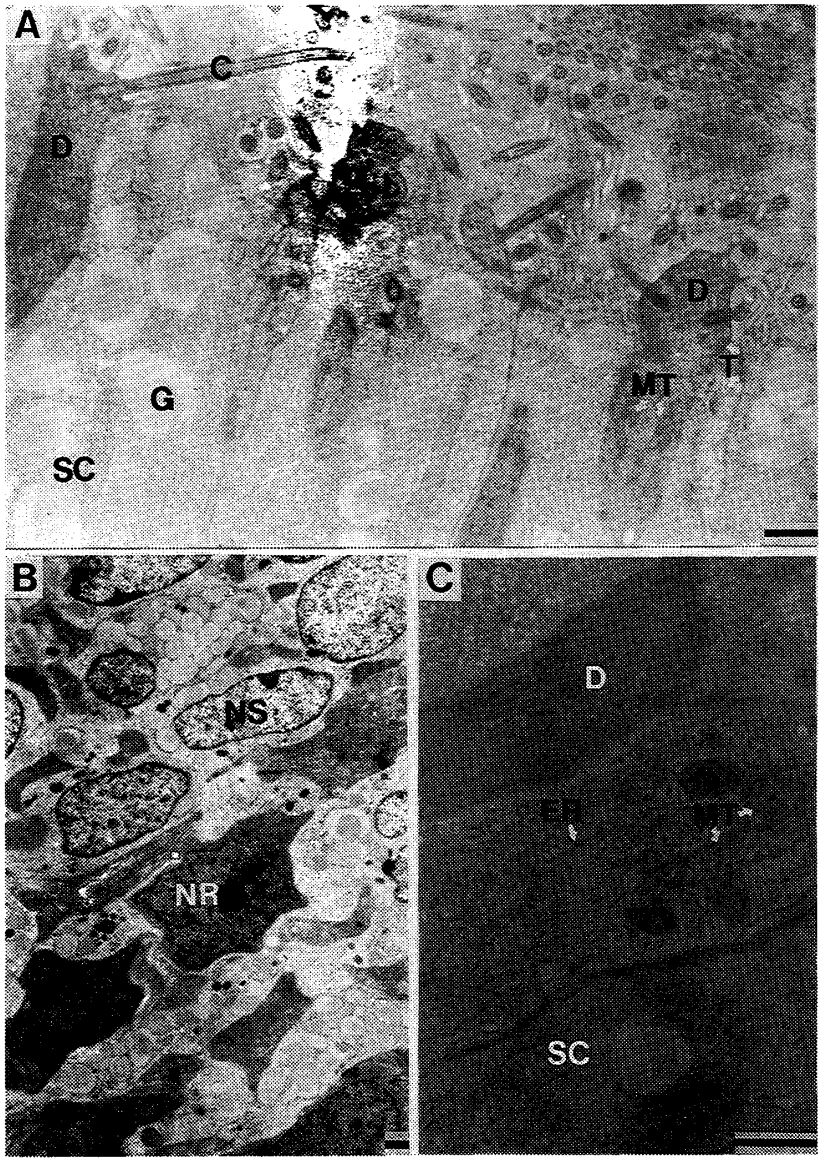

Fig. 2 Transverse sections of turtle olfactory mucosa.

A: Electron micrograph of distal part of the olfactory receptor. Receptor dendrite (D) swells and makes olfactory vesicle, where olfactory cilia (C) arose. Many mitochondria (MT) are seen in the dendrite. Supporting cell (SC) has microvilli and secretory granules (G). Junctional complexes (T) are observed near the swelling. Scale; 1 μm.

B: Nuclear layer of supporting cells and receptor cells. Below ovoid nuclei of supporting cells (NS), irregular shaped receptor cell nuclei (NR) with high electron density of cytoplasm are arranged. Scale; 1 μm.

C: Cytoplasm of supporting cells. Between the distal group and proximal group of secretory granules, thick mitochondria (MT) and rough surface endoplasmic reticulum (ER) are seen in the supporting cells (SC). Thin mitochondria are packed in high density receptor dendrite (D). Scale; 1 μm.

and supporting cell made junctional complexes near the dendrite swelling (Fig. 2-A).

In a micrograph sectioned parallel to the mucosal surface, cross sections of olfactory cilia and microvilli were observed. In the cross section of olfactory vesicle, some basal bodies were visible (Fig. 3-A). 4 - 6 dendrites of receptor cells surrounded one supporting cell, and each dendrite was separated one another by supporting cells. Junctional complexes between supporting cells or, supporting cell and receptor dendrite were observed in some places. In the supporting cells, secretary granules were seen. At slightly deep level, many cross sections of mitochondria were visible in the receptor dendrite. In a section parallel to the mucosal surface at the

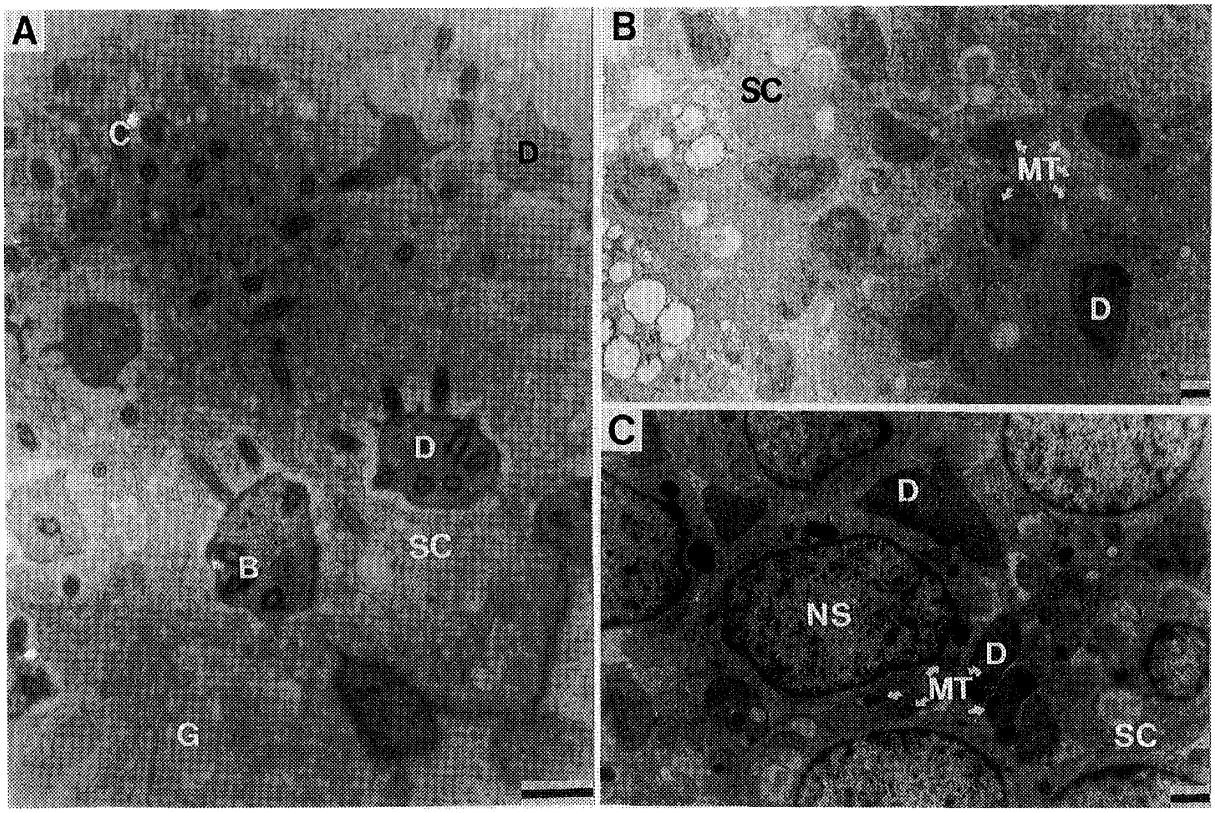

Fig. 3 Horizontal sections of turtle olfactory receptor mucosa.

A: Section parallel to the surface of the olfactory mucosa near surface. Receptor dendrites (D) which contain neurotubles and basal bodies (B) surround the supporting cell (SC). Secretory granules (G) are observed in the supporting cell. Scale; 1 μm.

B: Section parallel to the surface of the olfactory mucosa at the level of middle portion of supporting cell. Small round sections of mitochondria are packed in the receptor dendrite (D) with high electron density. Whereas thick sections of mitochondria (MT) are arranged around the outline in supporting cells (SC). Scale; 1 μm.

C: Section parallel to the surface of the olfactory mucosa at the level of supporting cell nuclei layer. Cross sections of mitochondria are observed boht in the receptor dendrite (D) and supporting cell perikaryon (SC). A few receptor dendrites are sometimes bundled. Scale; 1 μm.

level of central portion of supporting cells, arrangement of receptor dendrites surrounding a supporting cell became to be disturbed (Fig. 3-B). In the supporting cell, Golgi apparatus and rough surface endoplasmic reticulum were contained surrounded by large mitochondria which were about two times thicker than those in dendrites. In a section parallel to mucosal surface at the level of supporting cell nuclei layer, sometimes a few receptor dendrites were bundled and made direct contact (Fig. 3-C). In the cross section of dendrite, many mitochondria were visible. In the perikaryon of supporting cell, large mitochondria were still conspicuous.

Respiratory mucosa was composed with ciliated epithelial cell and goblet cell. The distal portion of the ciliated cell was elongated to 6 to 10 μm and scores or a hundred cilia which were 0.25-0.3 μm in thickness and 5 μm in length arose from there (Fig. 4-A). Moreover numerous microvilli with 0.1 μm thick and 2 - 3 μm long

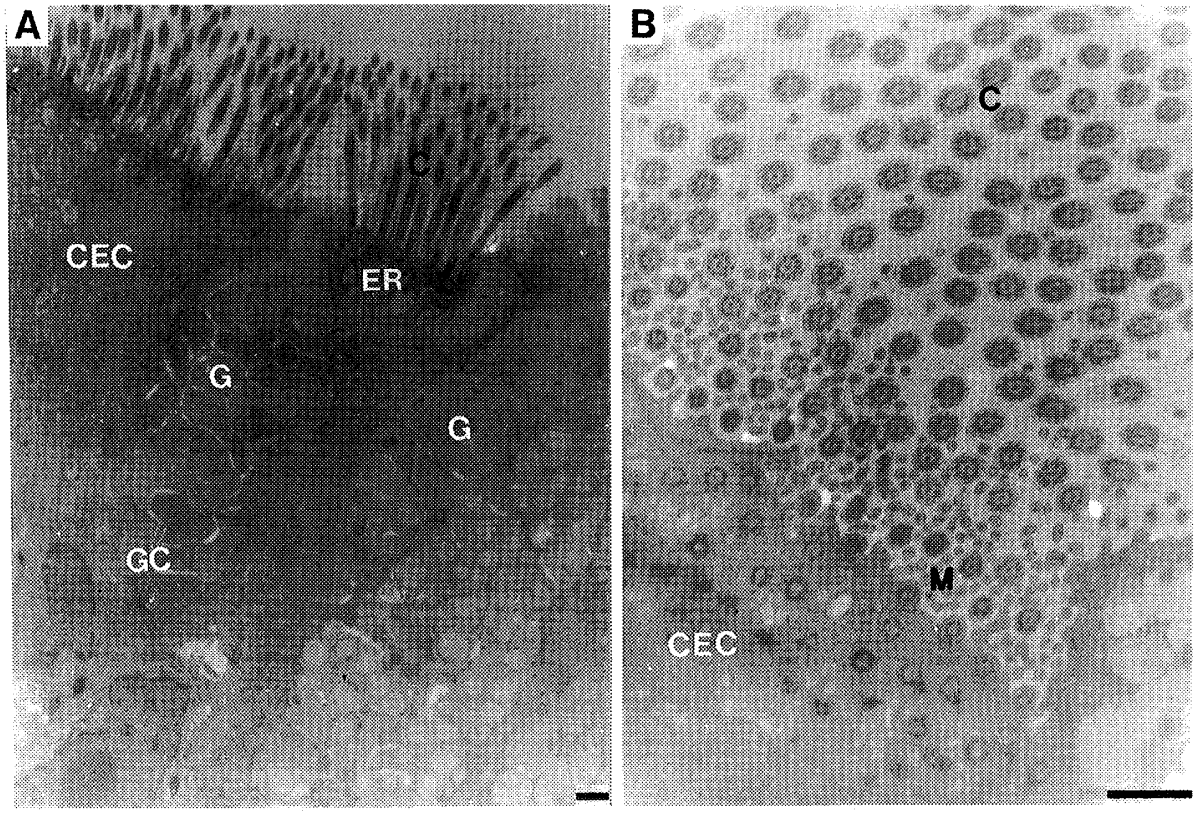

Fig. 4 Electron micrograph of the respiratory mucosa.
A: Distal portion of respiratory mucosal cells. Numerous cilia (C) are observed at the elongated distal portion of the ciliated epithelial cell (CEC). Small vesicle of smooth surface endoplasmic reticulum (ER) were contained there. In the goblet cell (GC), secretory granules (G) extend throughout the cell. Scale: 1 μm.
B: Section slighely oblique to the surface of the respiratory mucosa. Cross sectioned numerous cilia (C) and microvilli (M) of the ciliated cell are observed. Scale; 1 μm.

also arose between the cilia (Fig. 4-B). Many vesicles of smooth surface endoplasmic reticulum were scattered all over the cytoplasm. Nucleus of ciliated cell was ovoid and existed in relative shallow layer. Goblet cell was spindle shaped with 5 - 10 μm in diameter and a little cell surface exposed to the mucosal surface. Sometimes small microvilli were observed. Almost whole the soma, secretory granules with 1 - 2 μm in diameter occupied. In the secretory granule, high density or low density core were visible.

DISCUSSION

Ciliary type olfactory receptor cells were observed throughout vertebrate species (Engstrom et al., 1989; Kratzing, 1975; Okano et al., 1967; Reese, 1965). Whereas, microvillous olfactory receptor cells were observed in several species (Bannister, 1965; Ichikawa and Ueda, 1977; Yamamoto and Ueda, 1979). It was reported that there were some olfactory receptors which possessed both olfactory cilia and microvilli in newt (Usukura and Shibuya 1974). Olfactory receptors in Reeve's turtle also have cilia and microvilli like newt . Tufted short thick cilia of ciliated epithelial cells in respiratory mucosa shows synchronized beat to remove the mucus, and these cell have many smooth surface endoplasmic reticulum to control movement. On the other hands, very long olfactory cilia of receptor cells are entangled and piled up. It is therefore unlikely that cilia have motility (cilia seems to be motile). In fact, olfactory cilia in some species lacked dinein, the ciliary energy-transducing ATP ase (Lancet, 1986). So the main function of ciliary structure seems to be an enlargement of the receptor membrane. Olfactory binding protein, adenylate cyclass and associated ion channels are located on ciliary membrane, so olfactory transduction processed on ciliary membrane (Adamek et al., 1984; Nakamura and Gold, 1987). Vomeronasal receptor cells are microvillous type. So microvilli also possessed the olfactory transduction site. But, as yet, the study of microvilli as receptor membrane was not reported.

In Reeve's turtle, total area of receptor menbrane of olfactory receptor cells and that of vomeronasal receptor cells are determined, on the assumption that cilia and receptor cell have mean number of cilia or microvilli with mean size. The surface area of olfactory cilia was estimated at about 202 μm^2, while that of vomeronasal microvilli was estimated at about 138 μm^2. Microvillous vomeronasal receptor was by no means inferior than the ciliary olfactory receptors.

Two different results were reported about the functional difference between cilial and microvillous olfactory receptors. In catfish, the distribution of ciliary olfactory receptor cells and microvillous olfactory receptors differs, but there was no differences in responses specificities of ciliated and microvillous receptor for amino acids and bile salts (Erickson and Caprio, 1984).

Terrestrial newt have many cilial type of olfactory receptor cells, but when bred in water, the number of microvillous type olfactory receptor cells increased (Usukura & Shibuya, 1974). Moreover, corresponding with morphological changes, slow potential responses recorded from the olfactory mucosal surface, electro-olfactogram (EOG), to

vapor stimuli were reduced and those to aqueous stimuli were increased (Shibuya & Takagi, 1963; Arzt et al.,1986).

In several kinds of animals, that the vomeronasal organ could received non-volatile high molecules was proposed by electrophysiological or behavioral studies (Clancy et al., 1984; Hatanaka et al., 1988; Inouchi et al., 1989; O'Connell and Meredith, 1984). In Reeve's turtle, microvillous vomeronasal receptor and ciliated olfactory receptor received both volatile (airborn) and non volatile (aqueous) stimuli (Shoji et al., 1989). This could be concerned that the olfactory receptor have also microvilli, but it is reasonable to conclude that the specificity and ability of odor transduction between cilia and microvilli has no differences. It was known that the thickness of the cell coat varied between the cilia of the olfactory receptors and the microvilli of the vomeronasal recepotors (Mendoza and Breipohl, 1983). These differences should constrict the functional differences.

Olfactory cilia in aqueous fishes and catfishes were less crowded than that in reptiles, birds and mammals. Water born odor stimuli were solved in water and conveyed by water flow. So, as olfactory receptors in aqueous animals have microvilli or short cilia which don't disturb water flow and trap olfactory molecules. Generally. olfactory receptors of terrestrial animals receive only volatile chemicals from the air. Air as a medium is used till chemicals reach the mucosa. Odorant molecules must be dissolved into the mucus to reach receptive membranes (Hornung et al.; 1987). When the air mucus/partitioning of odorant occurs the concentration of odorant is weakened. It can be assumed that the olfactory receptors extended long cilia to the mucosal surface and make dense net for catching odorants molecules.

In the dendrite of olfactory receptor cells, and middle portion of supporting cell, numerous mitochondria are striking, but their size were different between two type cells. Large number of mitochondria in receptor dendrite indicate demand of large energy accompanying olfactory transduction or excitation. Increase of succinic dehydrogenase activity according to odor stimuli was detected at the dendrite region (Hatanaka and Kouno, unpublished). Those of supporting cell showed the energy supply to synthesize secretory granules. In the olfactory mucosa, mucus is secreted by Bowman's glands and supporting cells. Secretory granules in supporting cells of two kinds receptor mucosa and goblet cells of respiratory mucosa are not so different morphologically. But their constituent was not known. It is reported that secretion by supporting cells may be evoked by certain odorants. Olfactory mucus included soluble 'odorant binding protein' that bind and solubilize hydrophobic odorant in the mucus layer (Carr et al., 1990). This protein was not detected in the olfactory mucosa of aquatic vertebrates.

REFERENCES

Adamek, G.D., R.C. Gesteland, R.G. Mainr, and B. Oakley (1984) Transduction physiology of olfactory receptor cilia. Brain Res., 310:87-97.

Adams, D.R., and M.D. Wiekamp (1984) The canine vomeronasal organ. J. Anat.,

138:771-789.

Altner, H., and W. Müller (1968) Elektrophysiologische und elektronenmikroskopische Untersuchungen an der Riechschleimhaut des Jacobsonschen Organs von Eidechsen (*Lacerta*). Z. vergl. Physiol., 60:151-155.

Arzt, A.H., W.L. Silver, J.R. Mason, and L. Clark (1986) Olfactory responses of aquatic and terrestrial tiger salamander to airborne and waterborne stimuli. J. Comp. Physiol.,A 158:479-487.

Bannister, L. H. (1965) The fine structure of the olfactory surface of teleostean fishes. Quart. J. micro. Sci., 106:333-342.

Carr, W.E.S., R.A. Gleeson, and H.G. Trapido-Rosenthal (1990) The role of perireceptor events in chemosensory processes. TINS., 13:212-215.

Clancy, A.N., F. Macrides, A.G. Singer, and W.C. Agosta (1984) Male hamster copulatory responses to a high molecular weight fraction of vaginal discharge: effects of vomeroansal organ remove. Physiol. Behav., 33:653-660.

Engeström, B., A. Ekblom, and P. Hansson (1989) The olfactory and respiratory epithelium in rhesus and squirrel monkeys studied with freeze-fracture technique. Acta Otolarygol., 108:259-267.

Erickson, J.R. and J. Caprio (1984) The spatial distribution of ciliated and microvillous olfactory receptor neurons in the channel catfish is not matched by a differential specificity to amino acid and bile salt stimuli. Chemical Senses, 9:127-141.

Graziadei, P.P.C., and D. Tucker (1970) Vomeronasal receptors in turtles. Z. Zellforsch., 105:498-514.

Hatanaka, T., and T. Hanada (1986) Structure of the vomeronasal system and induced wave in the accessory olfactory bulb of red eared turtle. Proc. Jap. Symp. Taste and Smell, 20:183-186.

Hatanaka, T., O. Matsuzaki, and T. Shibuya (1982) Fine structure of vomeronasal receptor cells in the reeve's turtle, *Geoclemys reevesii*. Zool. Mag. (Tokyo), 91:190-193.

Hatanaka, T., T. Shibuya, and J. Inouchi (1988) Induced wave responses of the accessory olfactory bulb to odorants in two species of turtle, *Pseudemys scripta* and *Geoclemys reevesii*. Comp. Biochem. Physiol., 91A:377-385.

Hornung, D.E., S.L. Youngentob, and M.M. Mozell (1987) Olfactory mucosa/air partitioning of odorants. Brain Res., 413:147-154.

Ichikawa, M., and K. Ueda (1977) Fine structure of the olfactory epithelium in the goldfish, *Carassius auratus*. Cell Tiss. Res., 183:445-455.

Inouchi, J., J.L. Kubie, and M. Halpern (1989) Accessory olfactory bulb neurons in garter snakes respond to liquid delivery of odorants to vomeronasal organ. Proc. Jap. Symp. Taste and Smell, 23:87-90.

Kratzing, J.E. (1975) The fine structure of the olfactory and vomeronasal organs of a lizards (*Tiliqua scincoides scincoides*). Cell Tiss. Res., 156:239-252.

Lancet, D. (1986) Vertebrate olfactory reception. Ann. Rev. Neurosci., 9:329-355.

Matsuzaki, O., T. Shibuya, and T. Hatanaka (1982) Number of olfactory receptor cells and fine structure of olfactory nerve fibers in the pigeon, *Columbia livia*. Zool. Mag.

(Tokyo) 91:221-229.

Mendoza, A. S., and W. Breipohl (1983) The cell coat of the olfactory epithelium proper and vomeronasal neuroepithelium of the rat as revealed by means of the Ruthenium-red reaction. Cell Tiss. Res., 230:139-146.

Nakamura, T., and G. H. Gold (1987) A cyclic nucleotide gated conductance in olfactory cilia. Nature, 325:442-444.

O'Connell, R. J., and M. Meredith (1984) Effects of volatile and nonvolatile chemical signals on male sex behaviors mediated by the main and accessory olfactory systems. Behav. Neurosci., 98:1083-1093.

Okano, M., A. F. Weber, and S. P. Frommes (1967) Electron microscopic studies of the distal border of the canine olfactory epithelium. J. Ultrastruct. Res., 17:487-502.

Rees, T. S. (1965) Olfactory cilia in the frog. J. Cell Biol., 25:209-230.

Rowley Ⅲ, J. C., D. T. Moran, and B. W. Jafek (1989) Peroxidase backfills suggest the mammalian olfactory epithelium contains a second morphologically distinct class of bipolar sensory neuron : the microvillar cell. Brain Res., 502:387-400.

Shibuya, T., and S. F. Takagi (1963) Electrical response and growth of olfactory cilia of the olfactory epithelium of the newt in water and on land. J. Gen. Physiol., 47:71-82.

Shoji, J., M. Kashiwayanagi, and K. Kurihara (1989) Ionic permiability of turtle olfactory receptor membrane does not contribute to generation of receptor potentials. Proc. Jap. Symp. Taste and Smell, 23:75-78. (in Japanese)

Takami, S., and K. Hirosawa (1990) Electron microscopic observations on the vomeronasal sensory epithelium of a crotaline snake, *Trimeresurus flavoviridis*. J. Morphol., 205:45-61.

Usukura, J., and T. Shibuya (1974) Change in the fine structure of the olfactory receptor in newt. Zool. Mag. (Tokyo), 83:282. (in Japanese)

Vaccarezza, O. L., L. N. Sepich, and J. H. Tramezzani (1981) The vomeronasal organ of the rats. J. Anat., 132:167-185.

Wang, R. T., and M. Halpern (1980) Light and electron microscopic observations on the normal structure of the vomeronasal organ of garter snakes. J. Morphol., 164:47-67.

Yamamoto, M., and K. Ueda (1978) Comparative morphology of the fish olfactory epithelium. IX. Tetraodntiforms. Zool. Mag. (Tokyo), 88:210-213.