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Fine Structure of the Olfactory Epithelium in the Goldfish, *Carassius auratus*

A Study of Retrograde Degeneration*

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Summary. The fine structure of the goldfish olfactory epithelium was studied by transmission and scanning electron microscopy. Six different cell types were distinguished. Identification of the olfactory receptor cell was accomplished by use of retrograde degeneration studies. Two morphologically distinct types of olfactory receptor cells were identified: one type bears radially oriented cilia (Type I cell); the other type bears microvilli (Type II cell). The other four cell types were not identifiable as olfactory receptor cells: they are ciliated cells (Type III), rod-shaped cells (Type IV), supporting cells (Type V), and basal cells (Type VI).

Key words: Olfactory epithelium – Goldfish – Retrograde degeneration – Olfactory receptor cell – Electron microscopy.

Introduction

The fine structure of the olfactory epithelium of teleosts has been the subject of several previous studies. It is known that the olfactory epithelium of teleosts consists of three basic cell types: receptor cell, supporting cell, and basal cell (Trujillo-Cenoz, 1961; Bannister, 1965; Wilson and Westerman, 1967; Gemne and Døving, 1969; Schulte, 1972; Breipohl et al., 1973; Breipohl, 1974; Lowe and Macleod, 1975).

As for receptor cells, Bannister (1965) first reported in two species of teleosts (minnow and three-spined stickleback) three distinct types of receptor cells: (1) cells

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bearing cilia, (2) cells bearing microvilli, and (3) cells bearing a single simple rod. Three morphologically different receptor cells also have been reported in the eel (Schulte, 1972) and the goldfish (Breipohl et al., 1973). Lowe and Macleod (1975) reported two types of olfactory receptor cells in gadoid fish (cod, haddock): cells bearing cilia and others bearing microvilli. In crucian carp, however, Wilson and Westerman (1967) observed only one type of receptor cells, which was morphologically different from those described by Bannister (1965), Schulte (1972), and Breipohl et al. (1973). It is still not clear which one of these cell types is the actual olfactory receptor cell. Experimental evidence is needed to definitively identify the olfactory receptor cells.

The purpose of this study is to investigate the structural organization of the olfactory epithelium of goldfish and to determine by means of the retrograde degeneration technique which one of the cell types is the olfactory receptor cell.

Materials and Methods

Twenty-four goldfish *(Carassius auratus)* were used in these experiments. They measured 5 to 7 cm in length and were obtained from commercial sources. Transection of the olfactory nerve bundle was performed on one side between the olfactory organ and the olfactory bulb. On the other side the olfactory nerve bundle was left intact as a control. Three unoperated fish were also used as control. No morphological difference was found in the olfactory epithelia between the two controls. The operated fish were killed 2, 4, 6, 7, 10, or 14 days after surgery. The olfactory epithelium was immediately dissected out and immersed in a fixative containing 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2–7.4). It was then cut into small blocks. After being washed in 0.1 M sodium cacodylate buffer, the blocks were post-fixed with 1% OsO_4 in 0.1 M sodium cacodylate buffer for 2 hours. They were dehydrated in a graded series of ethanol.

The specimens for transmission electron microscopy were embedded in epoxy resin (TAAB embedding resin). Ultrathin sections cut with a Porter-Blum MT-2 ultramicrotome, were stained with uranyl acetate and lead citrate and examined with a Hitachi HS-9 transmission electron microscope.

For scanning electron microscopy, the dehydrated specimens were dried with liquid CO_2 using the Hitachi HCP-1 critical point dryer and coated with gold by use of the EIKO IB-3 ion coater. The specimens were studied with a Hitachi HHS-2R scanning electron microscope.

Results

Fine Structure of the Olfactory Epithelium

Six different kinds of cell types were distinguished in the olfactory epithelium of goldfish.

Type I. The cells of this type had an apical process which extended from the cell body toward the surface of the olfactory epithelium. The process contained 5 to 7 radially oriented cilia at its marginal end (Fig. 1). Nine pairs of outer tubules and 2 central ones (9+2 tubule structure) were characteristic of the cilia (Fig. 1a). The length of the cilia was about 7.0 μm. The diameter of the cilia was 0.23 ± 0.02 μm (Mean $\pm$ S.D.). At the attachment of cilia to the apical process there were basal bodies. The apical process was characterized by the presence of many longitudinally arranged microtubules, mitochondria, and other membranous structures. The cell

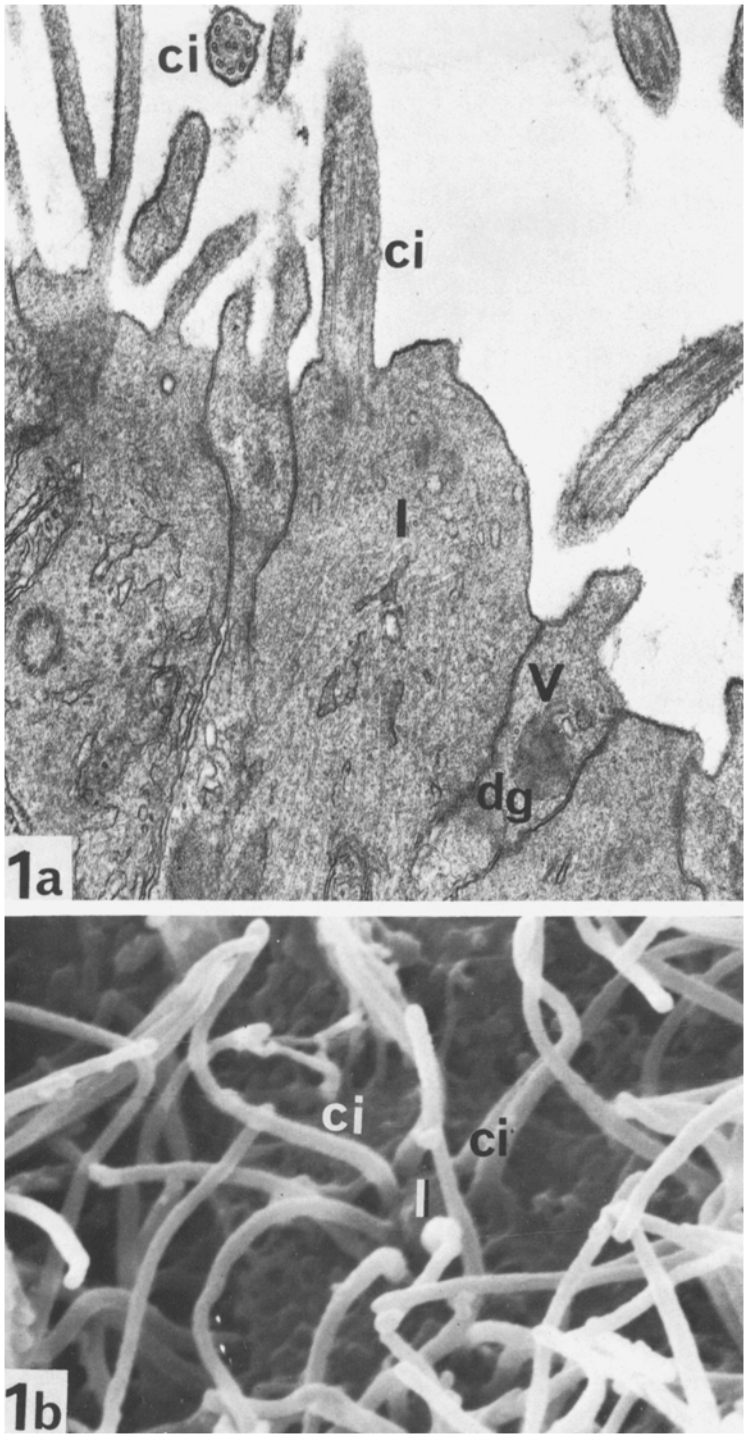


Fig. 1a and b. Photographs of the Type I cell. **a** Transmission electron micrograph showing cilia. **b** Scanning electron micrograph showing radially oriented cilia. *I* The Type I cell. *V* The Type V cell. *ci* cilium. *dg* dense granule. a) ×40,000; b) ×17,000

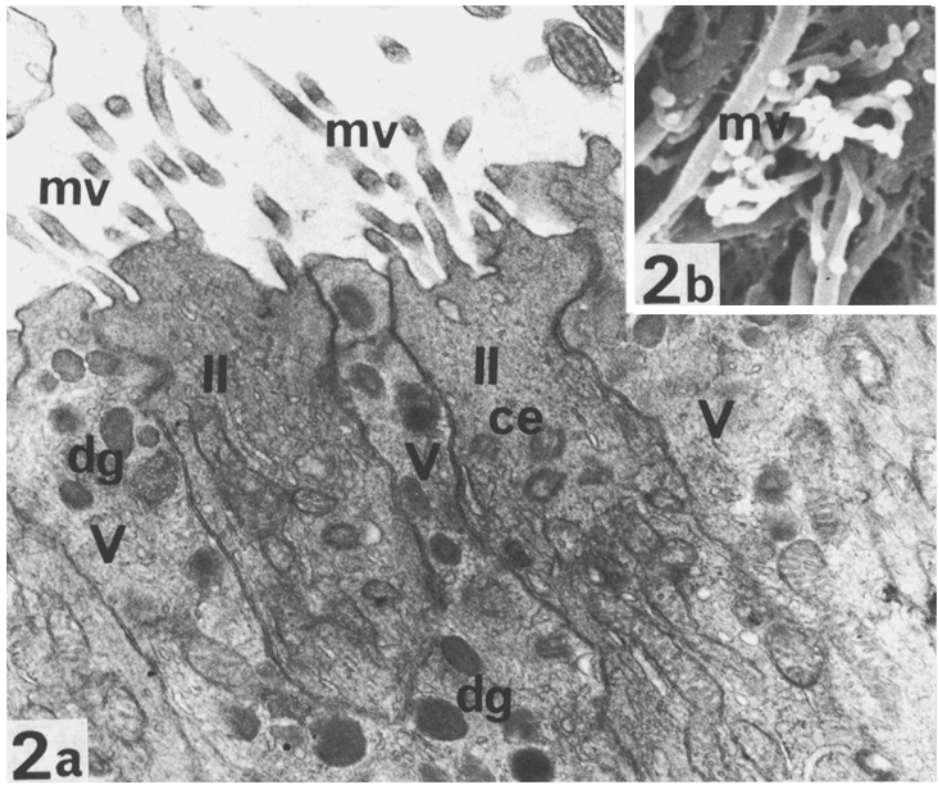


Fig. 2a and b. Photographs of the Type II cell. **a** Transmission electron micrograph showing microvilli (*mv*) and the position of the centriole (*ce*). **b** Scanning electron micrograph showing the microvilli. *II* The Type II cell. *V* The Type V cell. *dg* dense granule. a) ×20,000; b) ×17,000

body was spindle-shaped and contained an irregularly shaped nucleus. The cytoplasm of this cell type was electron-dense and contained mitochondria, rough endoplasmic reticulum, Golgi complexes, and free ribosomes.

Type II. The cells of this type had many microvilli on the superficial end of the apical process (Fig. 2). The number of microvilli ranged from 30 to 80. The length of the microvilli was about 2.0 μm. Their diameter was 0.08 ± 0.02 μm. The centriole was often observed in the apical process (Fig. 2a). Most other morphological features, except those specifically described above closely resembled the Type I cell.

Type III. The cells of this type showed a large number of cilia together with basal bodies and striated rootlets (Figs. 3–5). A (9 + 2) tubule structure was observed in these cilia. The length of the cilia was about 15 μm. The diameter was 0.18 ± 0.08 μm. Compound cilia in which several (9 + 2) tubular structures were surrounded by an outer membrane were occasionally observed (Fig. 4). Beneath the base of the cilia, many mitochondria were present (Fig. 3). In the electron-lucent cytoplasm, rough endoplasmic reticulum, Golgi complexes, and free ribosomes were observed. The

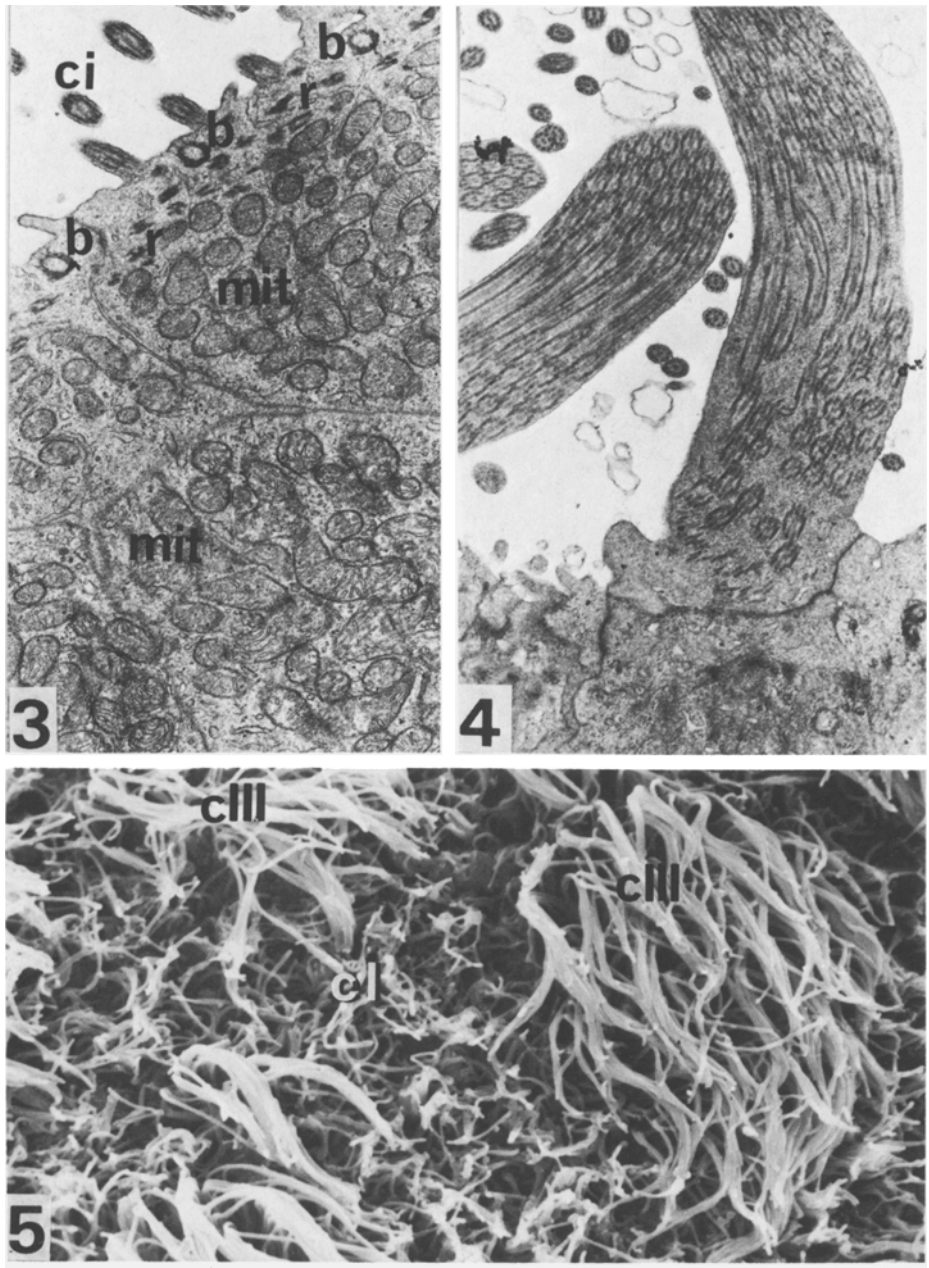


Fig. 3. Transmission electron micrograph of an oblique section of the olfactory epithelium. Photograph shows the Type III cell with cilia (*ci*) and many mitochondria (*mit*). Note that the plasma membranes of two adjoining cells of this type are closely apposed. *b* basal body. *r* basal rootlet. × 14,500

Fig. 4. Transmission electron micrograph showing a Type III cell with a compound cilium. Note the presence of several tubular structures. × 14,500

Fig. 5. Scanning electron micrograph of the olfactory epithelium. Photograph contrasts the cilia of Type I cell (*cI*) and Type III cell (*cIII*). × 3400

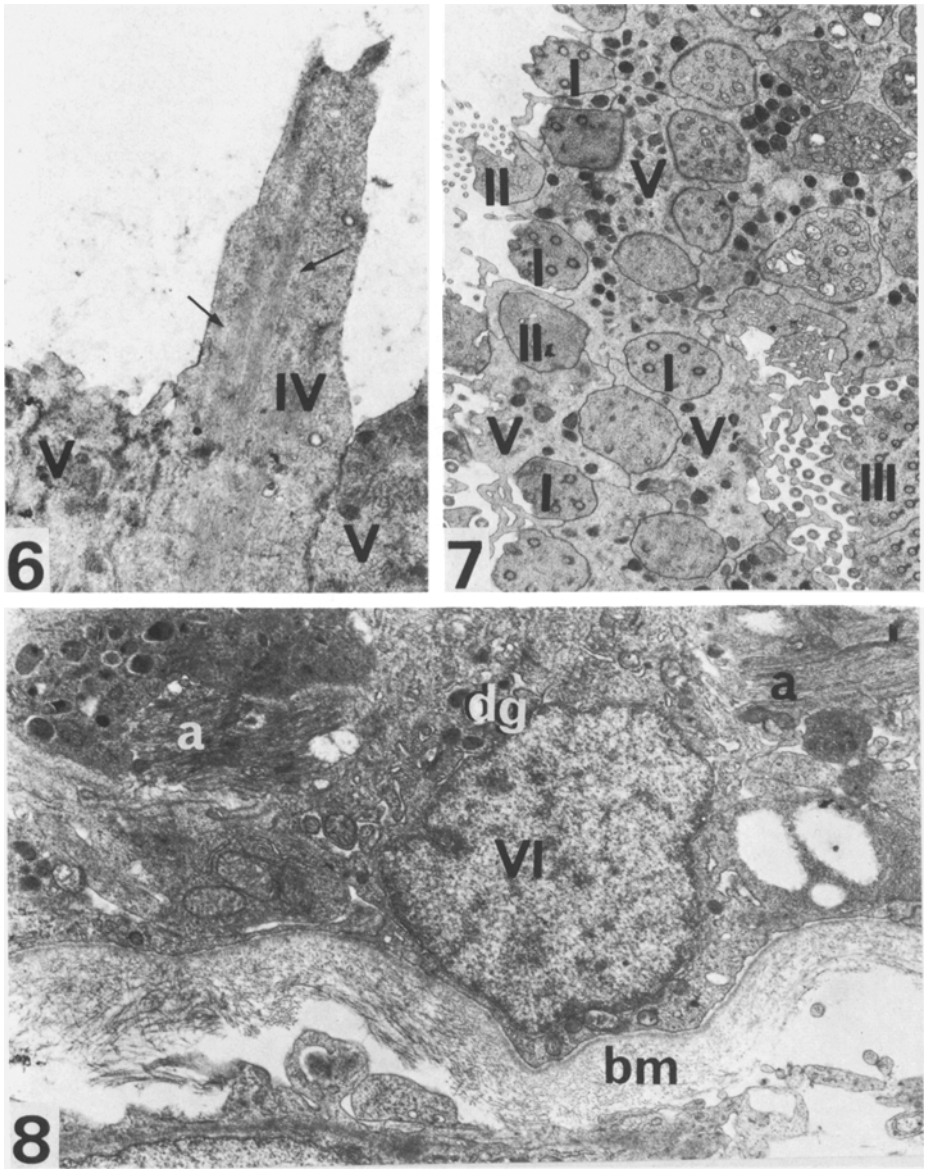


Fig. 6. Transmission electron micrograph of a Type IV cell, showing a rod-like cytoplasmic protrusion. Note the lack of cilia and microvilli. Arrows indicate bundle of filaments. *V* Type V cell. ×14,500

Fig. 7. Transmission electron micrograph of a section cut parallel to the plane of the free surface of the olfactory epithelium. Type V (*V*) cells with electron dense granules surround the cells of Types I (*I*) and II (*II*). *III* Type III cell. ×6000

Fig. 8. Transmission electron micrograph of the Type VI (*VI*) cell. *a* bundle of axons. *bm* basement membrane. *dg* dense granules. ×8500

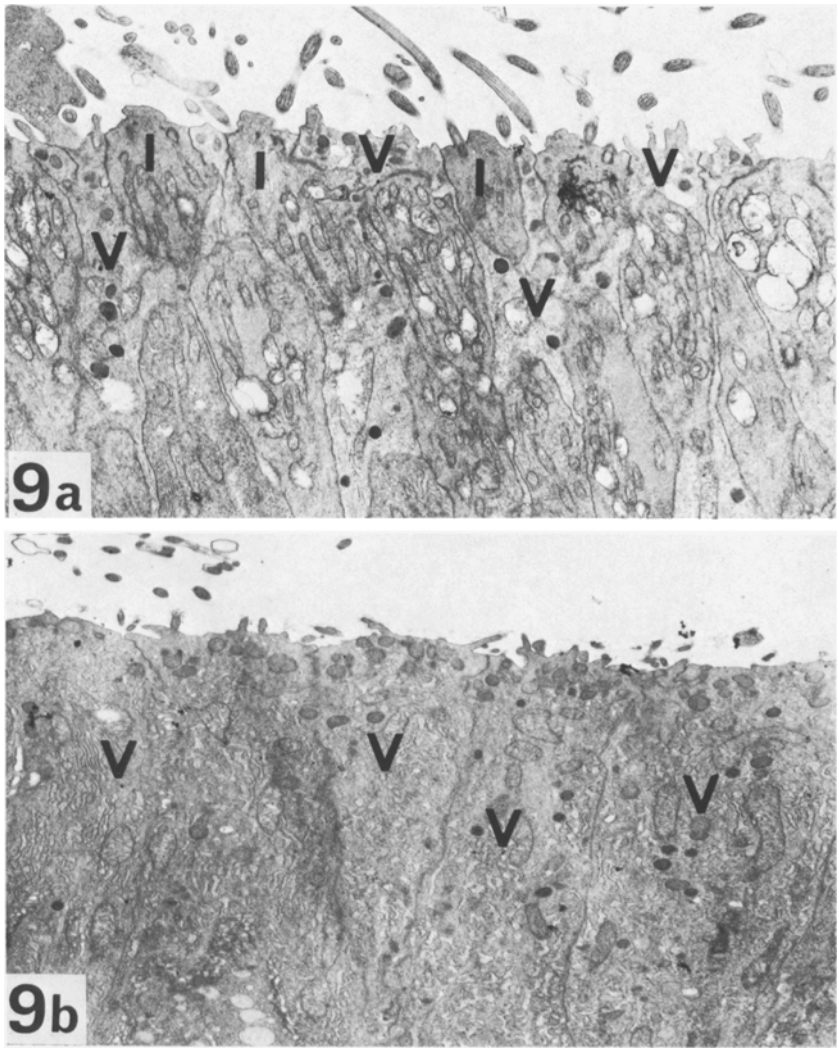
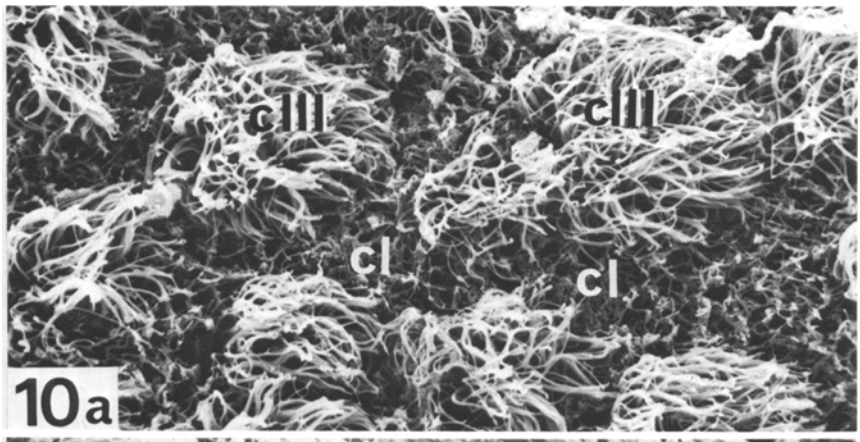


Fig. 9a and b. Transmission electron micrographs of the olfactory epithelium. **a** Intact epithelium. **b** Epithelium at 10 days after the transection of the olfactory nerve bundle. Note the absence of Type I and Type II cells in the photomicrograph (**b**). *I* Type I cell. *III* Type III cell. *V* Type V cell. a) and b) × 6000

nucleus was spherical in shape and lay at the base of cell. Five to seven cells of this type generally clustered together, and the plasma membranes of adjoining cells were closely apposed (Fig. 3).

Type IV. The cells of this type had neither cilia nor microvilli. Instead, a simple cytoplasmic rod projected from the free surface (Fig. 6). Bundles of closely packed filaments were observed in the rod-shaped structure (Fig. 6). This cell type was designated as "the rod-shaped cell" in Bannister's terminology (Bannister, 1965). Cells of this type were rarely observed in the goldfish.



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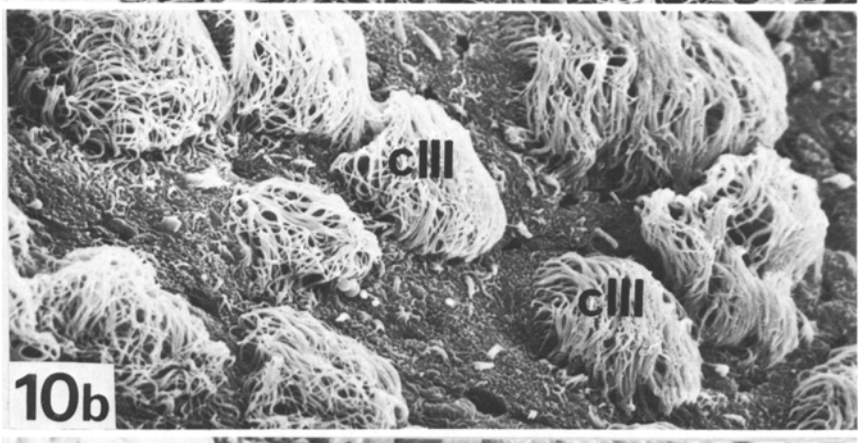

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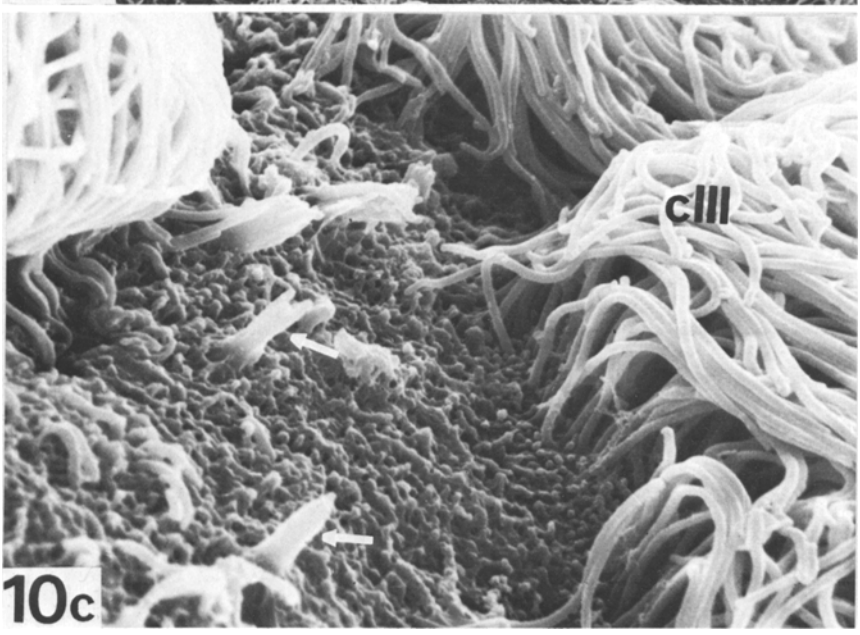

10c

Type V. The cells of this type were characterized by the presence of electron-dense granules in the cytoplasm. They surrounded Type I, II, and IV cells (Figs. 1a, 2a, 6, 7). Small protrusions were observed at the free surface of Type V cells (Figs. 1, 2, 7). Just beneath the free surface, junctional complexes with adjacent cells were observed. Moreover Golgi complexes, rough endoplasmic reticulum, mitochondria, and free ribosomes were observed.

Type VI. The cells of this type were adjacent to the basement membrane (Fig. 8). No cytoplasmic process extending from the cell body to the free surface was found. The shape of this cell was irregular. The cytoplasm contained rough endoplasmic reticulum, Golgi complexes, mitochondria, and free ribosomes. Electron-dense granules were often observed in the cytoplasm.

Identification of the Receptor Cells

In order to discriminate which one of the six types of olfactory epithelial cells are sensory receptor cells, a retrograde degeneration technique was applied. Degeneration patterns of specified cell types after transection of the olfactory nerve bundles were studied.

During the first 2 to 4 days after the surgery, there were no substantial morphological changes in the olfactory epithelium. At 6 or 7 days after surgery, most of the cells of Type I and II had degenerated and some had disappeared. The cytoplasm of the degenerated cells was electron-dense and vacuolated. The degenerated cells lacked cilia and microvilli. At 10 to 14 days after surgery, most of the cells of Type I and II had disappeared. The Type V cells occupied the positions where previously Type I and II cells were localized (Figs. 9, 10). The cilia of Type III cells were still visible. Type IV cells were also observed (Fig. 10).

In conclusion, effects of retrograde degeneration after transection of the olfactory nerve bundle were observed only in Type I and II cells, the other cell types appeared unaffected. For this reason, it appears that the Type I and II cells are the sensory olfactory receptor cells.

Discussion

In the present experiments, two morphologically different kinds of cells in the olfactory epithelium (Types I and II) were identified as receptor cells. The Type I cell is ciliated, while the Type II cell exhibits microvilli. These two types of cells have been described in other teleost fishes (Bannister, 1965; Schulte and Holl, 1971; Schulte, 1972; Lowe and Macleod, 1975). In goldfish, Breipohl et al. (1973) using the scanning electron microscope regarded the ciliated cells, the cells with microvilli and

Fig. 10a–c. Scanning electron micrographs of the olfactory epithelium. **a** Intact epithelium. **b** Epithelium at 10 days after the transection of the olfactory nerve bundle. Note the absence of the cilia of the Type I cells and the microvilli of Type II cells, but the presence of the cilia of Type III cells. **c** Higher magnification of (**b**). Arrow indicates the rod-shaped protrusion of Type IV cell. *cI* cilia of Type I cell. *cIII* cilia of Type III cell. a) and b) ×1700; c) ×8500

the cells bearing a rod-shaped protrusion as sensory receptor cells. Ciliated olfactory receptor cells are generally present in other vertebrates (Reese, 1965; Andres, 1966, 1969; Graziadei, 1966; Frisch, 1967; Graziadei and Bannister, 1967; Okano et al., 1967; Seifert and Ule, 1967; Thornhill, 1967).

The cell type which Wilson and Westerman (1967) considered to be the olfactory receptors in crucian carp was equivalent in structure to the Type III cell of the present paper. However, we were able to show in the lesioning experiment that the goldfish Type III cell is not responsive to nerve section and hence is not an olfactory receptor cell. Andres (1975) has suggested that the Type III cell of the goldfish is not an olfactory receptor cell. He proposed that its cilia are motile and might aid in the circulation of fluid across the olfactory lamella of the olfactory organ.

Bannister (1965), Schulte (1972), and Breipohl et. al. (1973) regarded the olfactory cell which bears a rod-shaped protrusion (Type IV cell in the present paper) as the olfactory receptor cell of teleosts. In our experiments, however, the rod-shaped cell (Type IV) was unaffected by olfactory nerve bundle transection. Therefore, the rod-shaped cell cannot be considered to be an olfactory receptor cell. The function of the rod-shaped cell (Type IV cell) could not be clarified by the experiments reported here. Type V cells and Type VI cells, respectively, may be regarded as supporting cells and basal cells, as already reported in other teleost fishes.

The significance of the cellular heterogeneity in the teleostean olfactory epithelium is not clear, but it may be suggested that the morphological differences may represent different progressive stages in differentiation of the receptor cells. Andres (1965, 1966) designated a 'blastema' cell type which might be a precursor of the receptor cell. In our study, obviously undifferentiated cells of the blastema type were not observed.

Graziadei and his co-workers have shown that the olfactory receptor cells of several vertebrates undergo a continuous turnover, and he suggested that the olfactory epithelium always consists of a mixture of immature, young, mature, aging, and degenerating receptor cells (Graziadei and Metcalf, 1971; Graziadei, 1973; Graziadei and DeHan, 1973). Thornhill (1967) also suggested that there is a turnover of receptor cells in the olfactory epithelium of lampreys. However, there is no evidence for such a turnover of the receptor cells in the present experiments.

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