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Fine Structure of the Lingual Dorsal Epithelium in the Bullfrog, *Rana catesbeiana*

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ABSTRACT—The structure of the lingual dorsal epithelial cells in the bullfrog, *Rana catesbeiana,* was investigated by light and transmission electron microscopy. Differences in the thickness of the epithelium were detected between the top and the base of each filiform papilla. Granular cells were located over all of the papillar epithelium, and these cells contained many electron-dense granules plus a few electron-lucent vacuoles, which may be a secretory form of the electron-dense granules in the cytoplasm on the free-surface side. There were a few mucus cells, the cytoplasm of which was filled almost completely with mucus granules. Ciliated cells were scattered among these granular cells.

INTRODUCTION

There have been many studies on the ultrastructure of the lingual taste organs or the sensory papillae of anurans [1–10]. However, of these reports, only a few describe aspects of the lingual epithelium other than the sensory papillae [8–10]. In particular, histological aspects of the lingual dorsal epithelium of anurans have been almost completely ignored.

In our recent study [8] using the scanning electron microscope, we showed that filiform papillae were distributed compactly over the entire dorsum of *Rana catesbeiana,* and that fine plicated structures, or microridges, were widely distributed on the surface of these papillar epithelial cells. In the present study, light and transmission electron microscopy were used to investigate the structure of the tongue of *Rana catesbeiana.*

MATERIALS AND METHODS

Five males and five females of the adult *Rana catesbeiana* were obtained commercially and used in the present study. Under MS-222 anesthesia, the animals were perfused from the heart with 1/2 Karnovsky solution which contained 2.5% glutaraldehyde and 2% paraformaldehyde in cacodylate buffer (pH 7.4). The tongues were then removed and refixed in the same solution for a few hr. After rinsing in 0.1 M cacodylate buffer, specimens were postfixed in phosphate-buffered 1% osmium tetroxide solution [11] at 4°C for 1.5 hr. This procedure was followed by dehydration, Epon-Araldite embedding, ultrathin sectioning, and U-Pb double staining. The specimens were then observed under a transmission electron microscope (Hitachi H-500, JEOL JEM-1200EX). Semi-thin sections of Epon-Araldite embedded specimens were stained with 0.2% toluidine blue in 2.5% Na_2CO_3. Micrographs of the sections taken with a light microscope (Olympus BH-2) were compared with the transmission electron micrographs.

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RESULTS

The epithelium which forms the upper part of the filiform papillae was revealed by light microscopy to be thicker than that forming the basal part. The former was composed of stratified columnar cells, while the latter was composed of simple columnar and/or cuboidal cells. In the upper region of the filiform papillae, a relatively large number of mucus cells were seen in comparison to the basal part of the filiform papillae. The connective tissue and the smooth muscle penetrated deeply into the center of each papilla (Fig. 1).

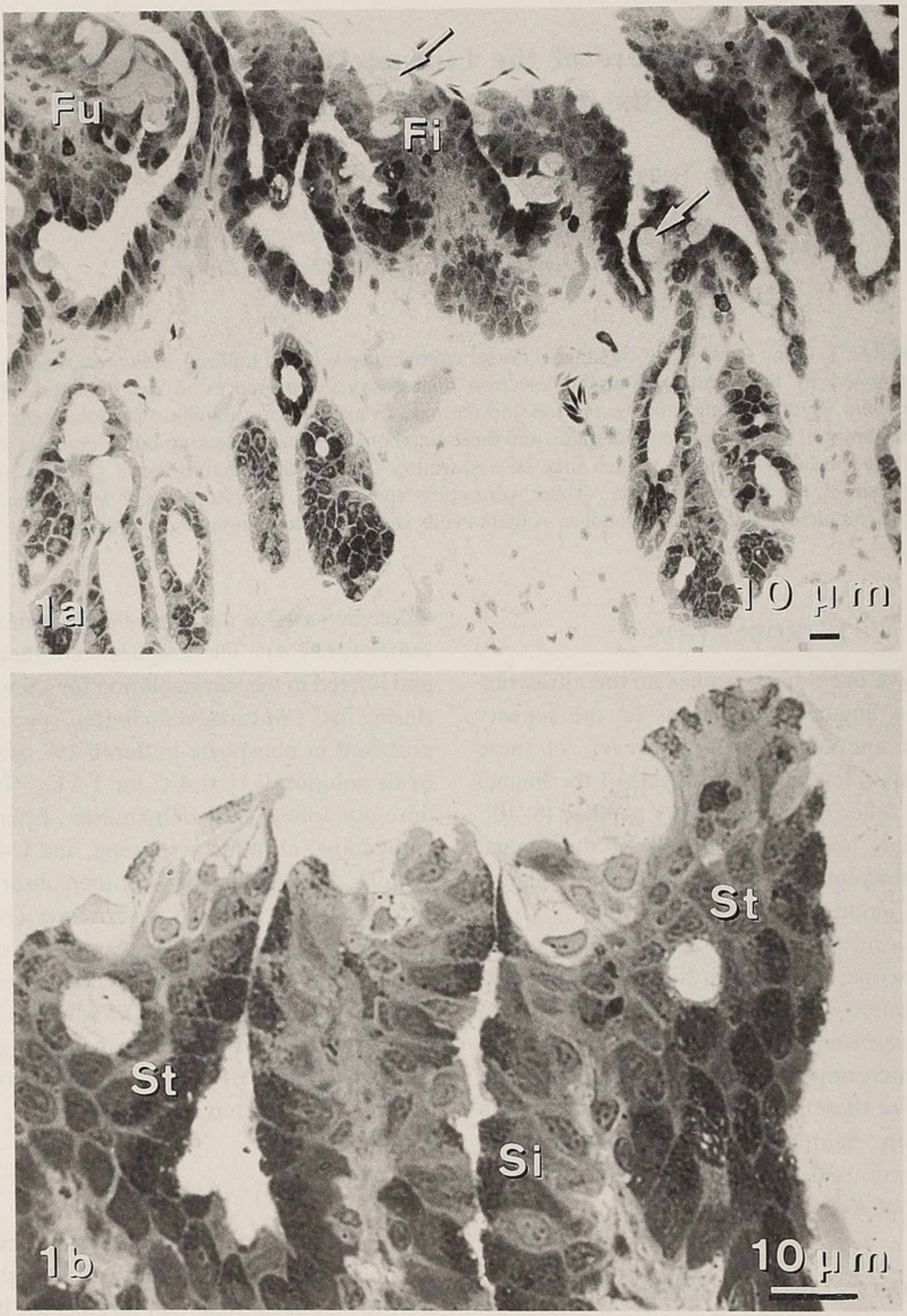


Fig. 1. Light micrograph of the lingual dorsal mucosa of the frog, *Rana catesbeiana,* embedded in Epon-Araldite. a. Filiform papillae (Fi) and fungiform papilla (Fu) are observed. Arrows show mucus cells. A large portion of the epithelium is composed of granular cells and ciliated cells, but they are not distinguishable from each other at this magnification. b. The upper area of filiform papillae. A large part of the top area of papillar epithelium consists of stratified columnar cells (St). The rest consists of simple columnar and/or cuboidal cells (Si).

Transmission electron microscopy of the stratified columnar epithelium in the upper portion of the filiform papillae revealed the presence of cells that contained a large number of electron-dense, round granules (Fig. 2). Some of the cells not only contained these round granules but also a few, electron-lucent vacuoles. The nucleus was located basally, and rough-surfaced endoplasmic reticulum was well-developed in the perikaryon (Fig. 2). The columnar and/or cuboidal cells of the epithelium at the base of the filiform papillae also contained a large number of electron-dense granules. The same cells often contained a small number of electron-lucent vacuoles. The nucleus was located basally, and rough-surfaced endoplasmic reticulum was well-developed in the perikaryon. In both the upper and the basal regions of the filiform papillae, microridges were widely distributed on the free-surface side of the epithelial cell. The cell surfaces which faced adjacent cells bore abundant cellular processes (Fig. 2). At higher magnifaction, the electron-dense granules were observed to consist of randomly packed tubules, or in some instances, appeared to show polygonal packing. The diameter of each tubule was 30–40 mμ. Endoplasmic reticulum and free ribosomes were dispersed in the cytoplasm around the electron-dense granules (Fig. 3). In some cases, an image showing that the electron-lucent vacuole seemed to take in electron-dense granules was observed. The tubular structures in the electron-dense granules might be lost in this process (Fig. 4). In some granular cells, the contents of the electron-lucent vacuoles were likely to be lost by exocytosis (Fig. 5).

Large cells, in which almost all the cytoplasm was filled with mucus, were scattered among the electron-dense granular cells (Fig. 6). There were more of these large mucus cells in the upper portion of the filiform papillae than in the base (Fig. 1)

Ciliated cells were scattered among the granular cells in the filiform papillar epithelium. The ciliated cells had cilia and microvilli on their free exterior surfaces. The nucleus was located basally. Just beneath the free surface of the cells, basal bodies could be recognized. Ciliary rootlets were distributed throughout the cytoplasm. Free ribosomes and endoplasmic reticulum were seen mainly in the perikaryon (Figs. 7 and 8).

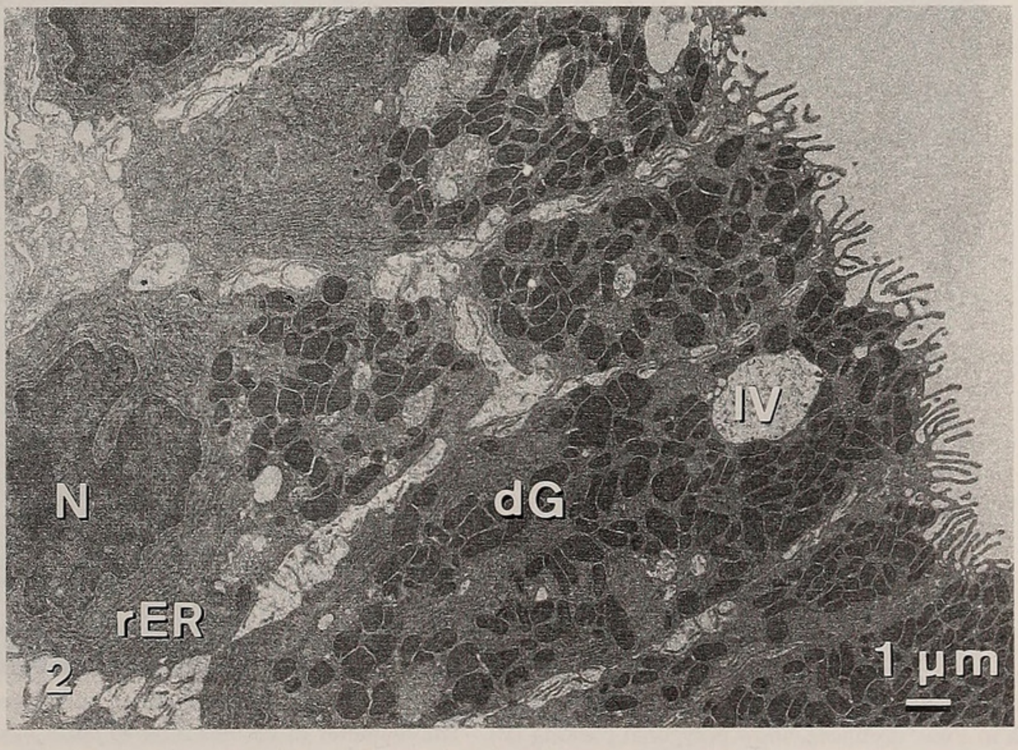
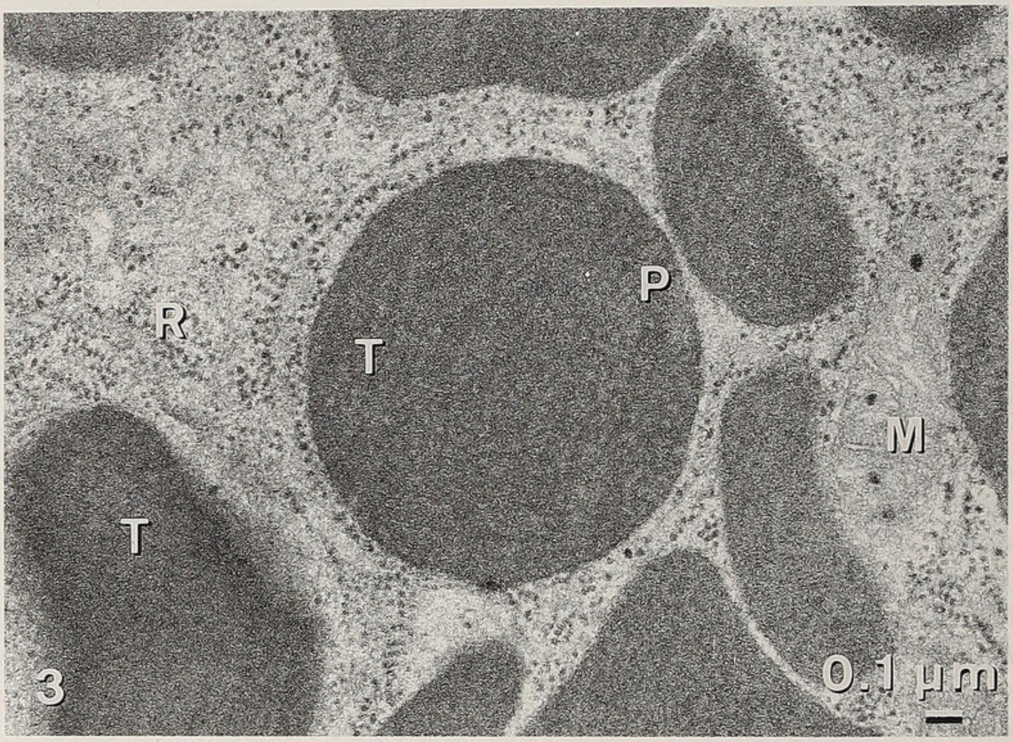


FIG. 2. Transmission electron micrograph of the epithelial cells in the upper part of a lingual filiform papilla from *R. catesbeiana*. Nucleus (N) is located basally. Rough-surfaced endoplasmic reticulum (rER) is well-developed in the perikaryon. A large part of the cytoplasm of each cell is occupied by many electron-dense granules (dG). An electron-lucent vacuole (lV), which seems to be the secretory form of electron-dense granules, is seen just beneath the free-surface of the cell.



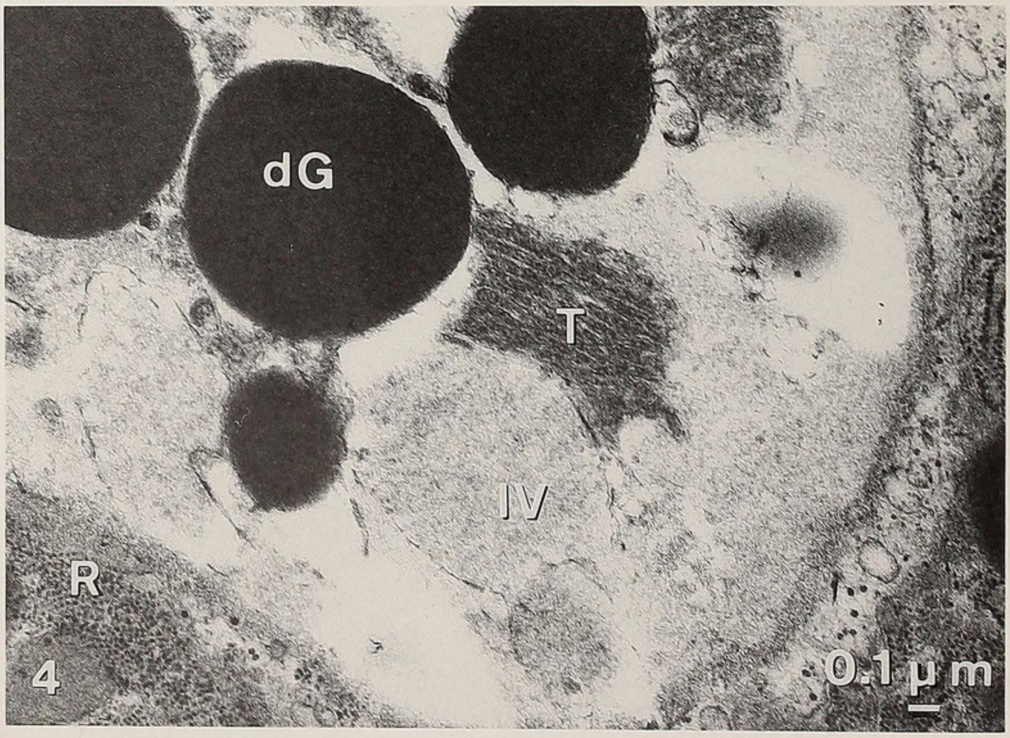
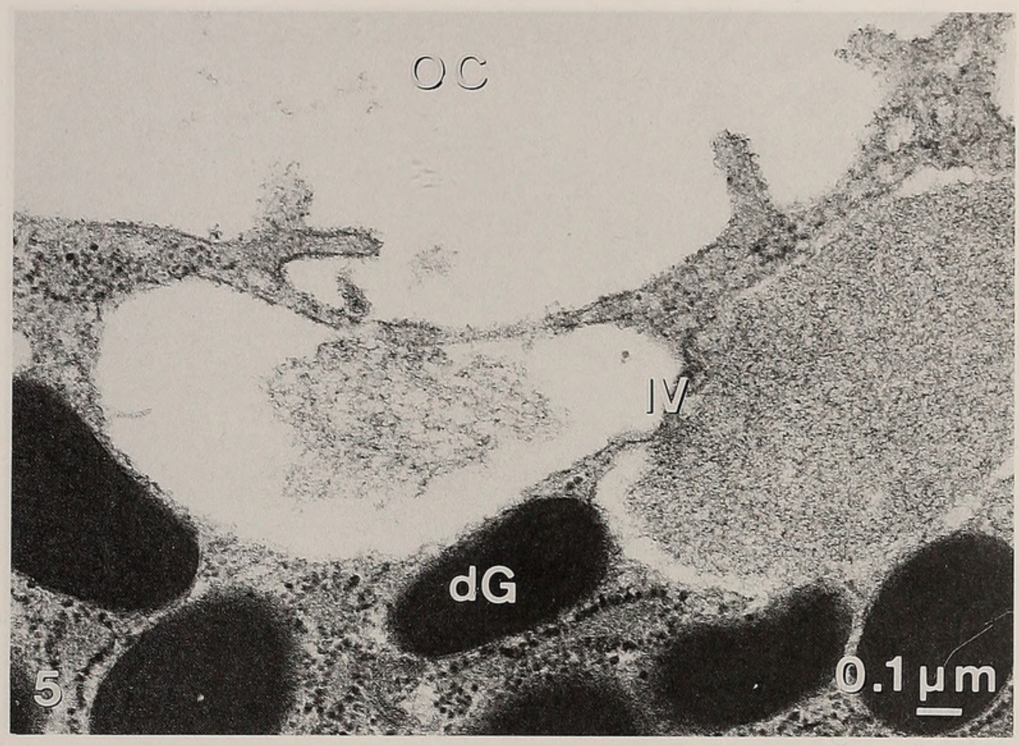


Fig. 3. Higher magnification of electron-dense granules. These granules consist of randomly packaged tubules (T) which in some instances, show polygonal packing (P). The diameter of tubules is 30-40 μm.

Fig. 4. Transmission electron micrograph showing the process in which the electron-lucent vacuole (lV) seems to take in the electron-dense granules (dG). T: tubular structure of the collapsing electron-dense granules, R: ribosomes.



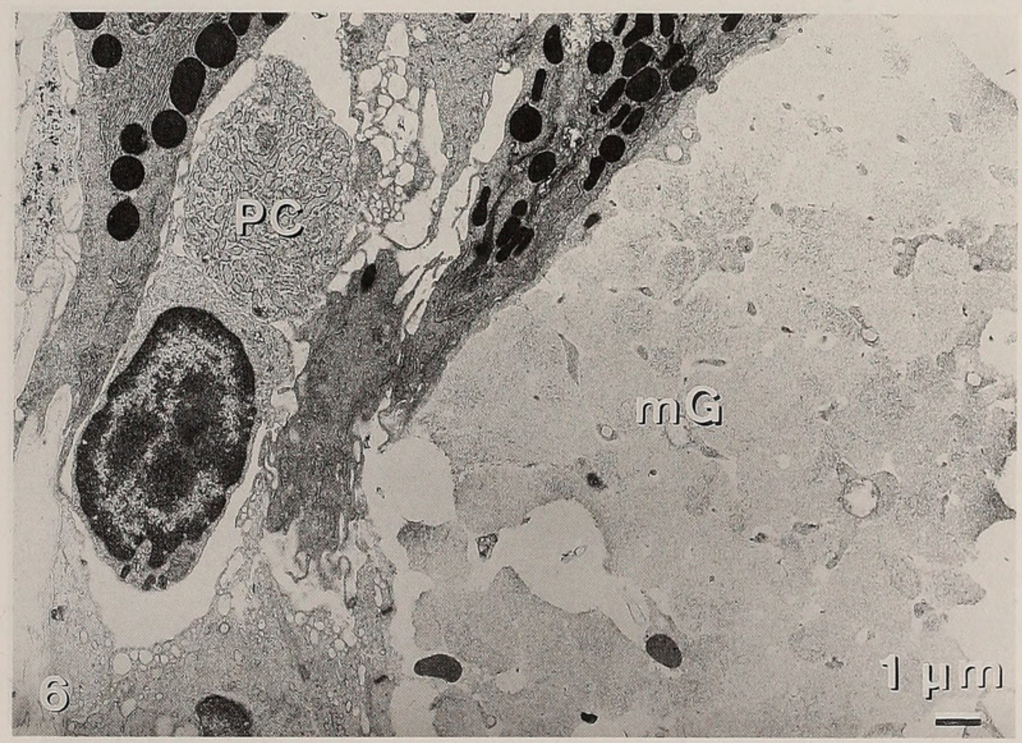
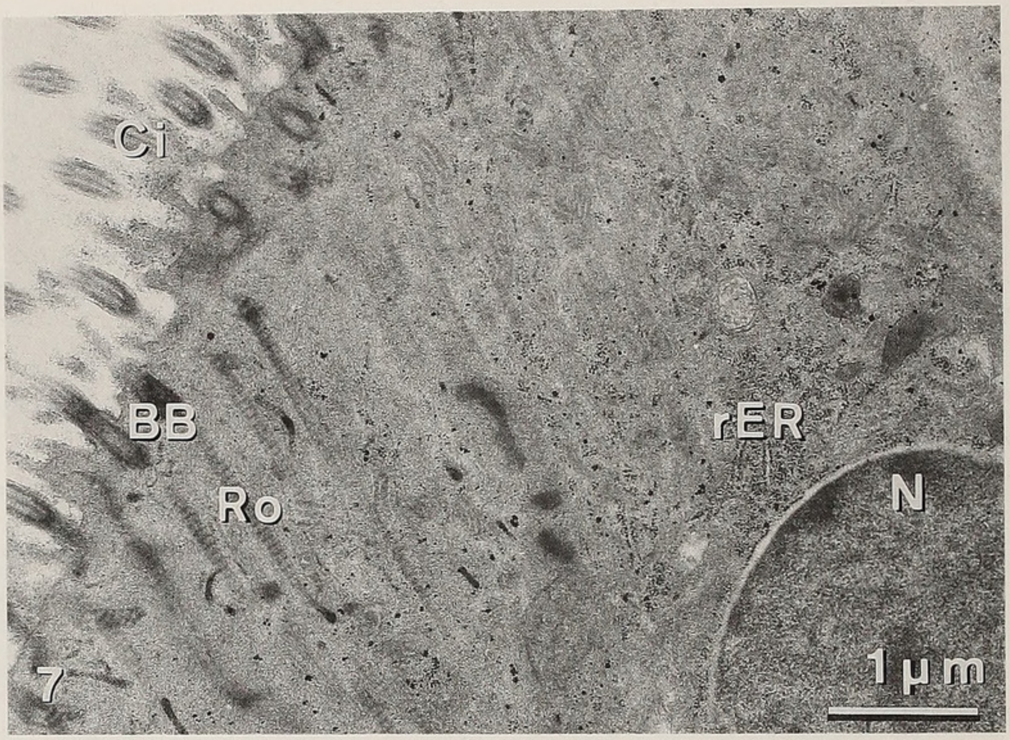


FIG. 5. Transmission electron micrograph of electron-lucent vacuoles (lV) just beneath the free surface of the cell. The contents of vacuoles were likely to be discharged into the oral cavity (OC) by exocytosis. dG: electron-dense granule.

FIG. 6. Transmission electron micrograph of a mucus cell; almost all the cytoplasm is filled with mucus granules (mG) and a plasma cell (PC).

On very rare occasions, cells were observed which originated from the epithelium but which lacked electron-dense granules, mucus granules, and cilia (Fig. 9). In these cells, the nucleus was basally located, and mitochondria, free ribosomes, and glycogen granules were abundantly scattered



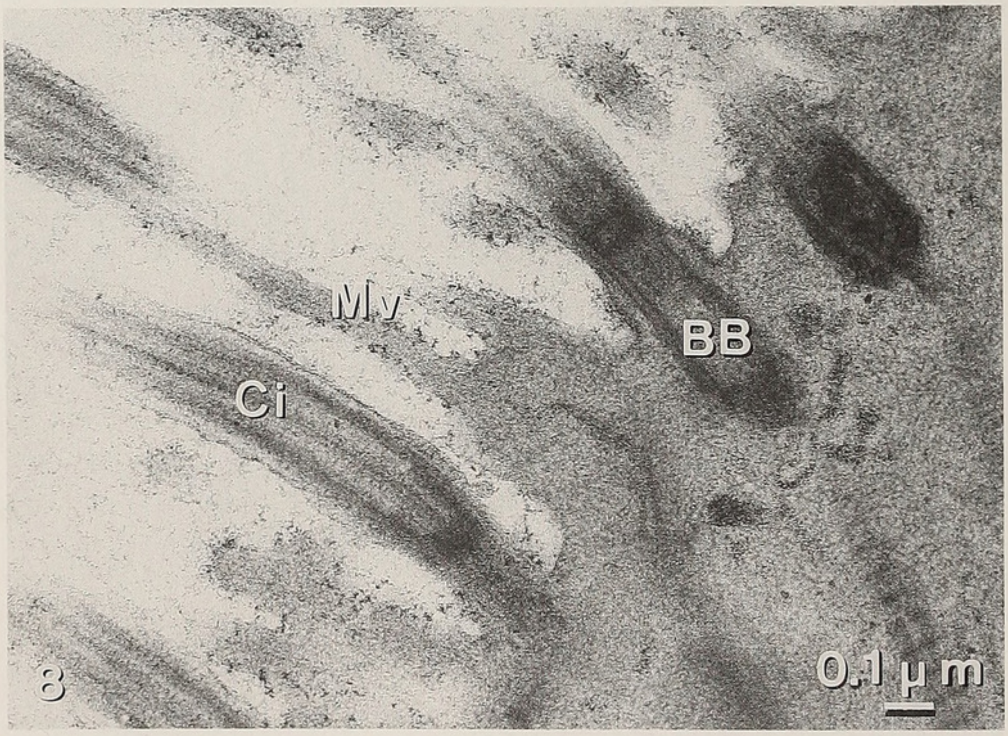
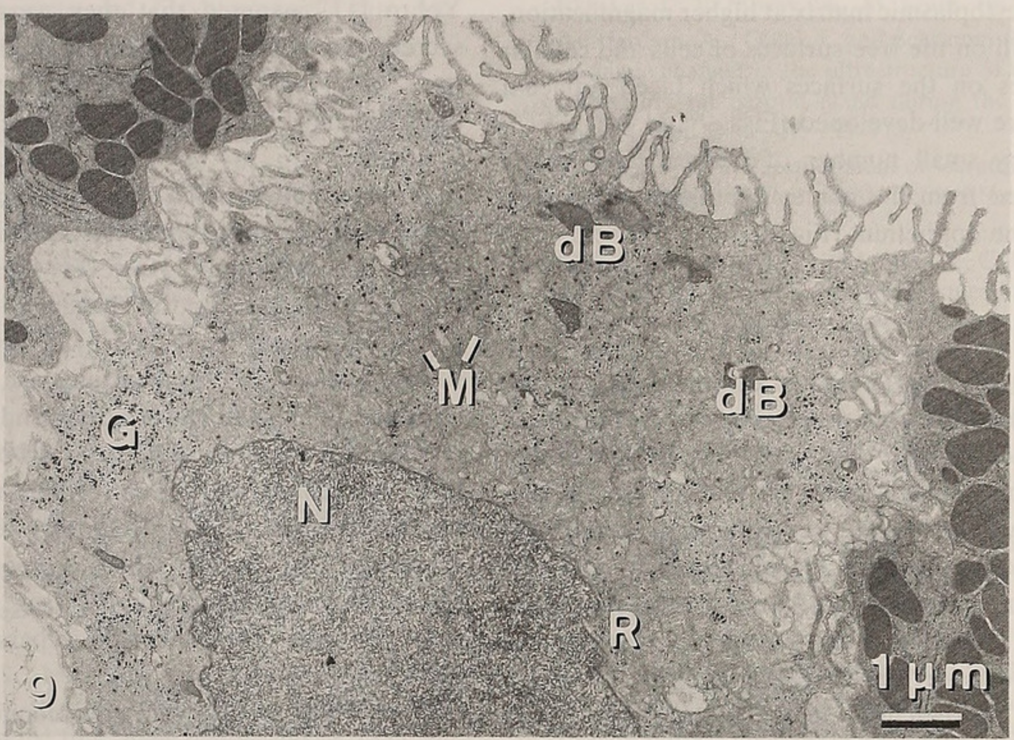


FIG. 7. Transmission electron micrograph of a ciliated cell. Nucleus (N) is located basally. Basal bodies (BB) can be recognized just beneath the free-surface of the cell. Ciliary rootlets (Ro) are distributed in the cytoplasm. Rough-surfaced endoplasmic reticulum (rER) are seen mainly in the perikaryon. Ci: cilia.

FIG. 8. Higher magnification of the free-surface side of a ciliated cell. Microvilli (Mv) are scattered among cilia (Ci). BB: basal bodies.

in the cytoplasm (Fig. 10). The mitochondria contained some intramitochondrial granules. A few electron-dense bodies, which were probably the basal bodies, were present within the cells on the free-surface side in the cytoplasm. Filamentous structures were recognized through-



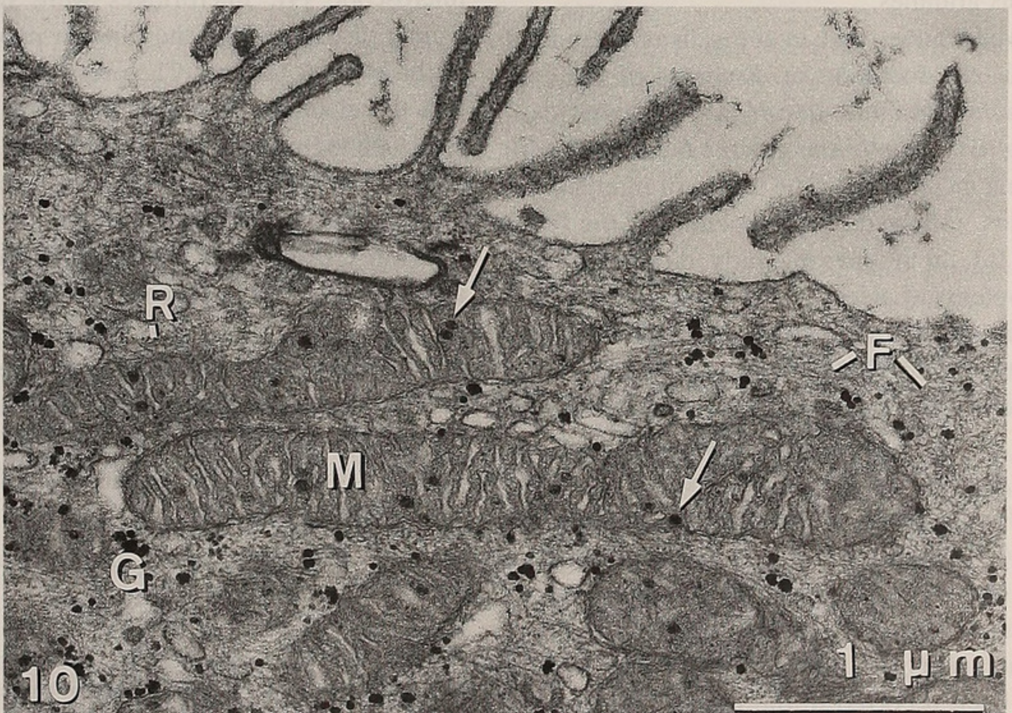


FIG. 9. Transmission electron micrograph of a cell without electron-dense granules, mucus granules, or cilia. The nucleus (N) is located basally. Mitochondria (M) and glycogen granules (G) are distributed throughout the cytoplasm. Free ribosomes (R) are seen mainly in the perikaryon. Dense bodies (dB) are located on the free-surface side.

FIG. 10. Higher magnification of the free-surface side of the cytoplasm of a cell which contains no electron-dense granules, mucus granules, or cilia. Mitochondria (M) contain intramitochondrial granules (arrows). Glycogen granules (G) and free ribosomes (R) can be recognized in the cytoplasm. Filamentous structures (F) are seen throughout the cytoplasm.

out the cytoplasmic matrix at higher magnification. Microvilli on the free-surfaces of cells and cellular processes on the surfaces which faced adjacent cells were well-developed (Figs. 9 and 10).

A very small number of plasma cells, which originated from the connective tissue, were seen within the epithelium (Fig. 6).

DISCUSSION

There are two possible explanations for the presence of electron-dense granules in the whole papillar epithelium: one is that they may be immature forms of mucus granules, and the other is that they may be analogous to serous granules found in the salivary glands of higher organisms [12–14]. However, it appears that the electron-dense granules are unrelated to the mucus granules because we found no evidence of any transitional stages existing between the elecron-dense granules and the mucus granules.

It is well known that in the salivary glands of mammals, the secretion or discharge of the contents of serous granules occurs through exocytosis, involving the fusion of the granule membrane with the plasma membrane at the lumen or intercellular canaliculus, followed by the opening of the fused portion [15]. In the present study, direct discharge of the contents of electron-dense granules into the oral cavity was not obvious. Instead, it seems likely that after some electron-dense granules aggregate to form an electron-lucent vacuoles, the contents of the electron-lucent vacuoles are discharged into the oral cavity by exocytosis. This scheme for secretion of the contents of the granules is very similar to the rapid secretion of the contents of serous granules in the salivary glands of mammals, as it occurs after stimulation with pharmacologic agents [15, 16].

It has been reported in the mouse [17] and the rat [18], that secretory granules of the submandibular gland of newborns contain tubular structures. In the present study of the bullfrog tongue, we found that the secretory granules of the cells within the lingual dorsal epithelium contained tubular structures similar to those found in the submandibular glands of newborn mice. Based on the time at which the tubular structures appear, Yohro [17] assumed that they were related to secretory activity. Therefore, the analogous structures observed in the lingual epithelial cells of bullfrogs may be involved in the active secretion of granules.

A very small number of cells without electron-dense granules, mucus granules, or cilia was found within the epithelium. However, these cells contain a lot of mitochondria throughout their cytoplasm, and have many microvilli on their free-surface. These structural features are very similar to those of the flask cells or mitochondria-rich cells [19–21]. The mitochondria-rich cells of amphibian skin epithelium have been implicated in the mechanism for passive chloride conductance across the skin [22–24]. There is a possibility that the mucosal epithelium of the frog tongue also plays the same role as the skin in passive chloride conductance.

In previous studies with the scanning electron microscope [9], microridges were found to be widely distributed on the filiform papillar surface of the bullfrog tongue. The present study indicates that the electron-dense granular cells have microridges on their free-surfaces, and cellular processes [25] on the surfaces which face adjacent cells. There are no significant differences between these structures except for whether or not they face adjacent cells. By scanning electron microscopic observation [9], they are clearly different from microvilli. These cellular processes seem to function as connecting structures between adjacent cells, while the microridges on the free-surface probably facilitate the spreading and holding of mucus [26].

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