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Fine Structure of the Lingual Dorsal Epithelium of the Japanese Toad, *Bufo japonicus* (Anura: Bufonidae)

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ABSTRACT—The structure of the so-called "ridge-like papillae" of the lingual dorsal epithelium of the Japanese toad, *Bufo japonicus* was investigated by light and transmission electron microscopy. The top of each ridge-like papilla was composed of stratified columnar epithelium and its base was composed of simple columnar epithelium. The apical cells, which were located on the top of ridge-like papillae, contained a large number of mitochondria, free ribosomes and fibrous structures, and a small number of electron-dense, round granules and clear bodies. The nuclei were located in the basal part of apical cells. By contrast, cells that contained a large number of mucus granules were located at the base of the papillae. The nuclei were located in the basal part of cells. Rough-surfaced endoplasmic reticulum and Golgi apparatus were distributed in the perikaryal part of cytoplasm. Ciliated cells were also scattered at the base of the papillae. The nucleus was located in the basal region of these cells. Mitochondria and ribosomes were widely distributed in the cytoplasm. Basal bodies could be seen just beneath the free surface of the cells. In addition to these cells, a very small number of mitochondria-rich cells were located within the epithelium. Distinct, fine, cellular protrusions were distributed in a compact array on the free surface of these cells.

INTRODUCTION

We showed in our recent study [1], using the scanning electron microscope, that irregular, undulant structures or ridge-like papillae, which may be homologous to the filiform papillae of frogs, are distributed compactly over the entire dorsum of the tongue of *Bufo japonicus*. We also demonstrated that fine, plicated structures or microridges are widely distributed over the surface of these papillar epithelial cells. Furthermore, in *Bufo japonicus,* no ciliated cells were observed on the surface of the ridge-like papillae or in the surrounding areas of the sensory disc [1], while in *Rana,* many ciliated cells were seen on the surface of the filiform papillae and in the surrounding areas of the sensory discs [2, 3]. The purpose of the present study was to clarify the histological and cytological structure of the lingual epithelium of *Bufo japonicus* and to compare the results to those for *Rana* [4, 5]. Light and transmission electron microscopy were used for this purpose.

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MATERIALS AND METHODS

Five male and five female adult specimens of *Bufo japonicus* were obtained commercially and used in the present study. Under MS-222 anesthesia, the animals were perfused from the heart with 50% diluted Karnovsky solution that 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 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 or JEOL JEM-1200EX). Thick sections from the blocks embedded in Epon-Araldite were stained with 0.2% toluidine blue in 2.5% Na_2CO_3. Micrographs of sections taken under a light microscope (Olympus

BH-2), were compared with the transmission electron micrographs.

RESULTS

The epithelium at the tip of the ridge-like papillae was thicker than that in the basal part, as revealed by light microscopy of sections of Epon-Araldite embedded material. The apical epithelium was composed of stratified columnar cells, whereas the basal epithelium was composed of simple columnar cells. Mucus granules, which stained densely with toluidine blue, were concentrated mainly at the base of the papillae and occupied about 60% to 70% of the epithelium. Connective tissue and smooth muscle penetrated deeply into the center of each papilla (Fig. 1).

Transmission elecron microscopy of the stratified columnar epithelium in the apical portion of the papillae revealed apical cells which contained a small number of elecron-dense, round granules just beneath the free surface. The size of each granule varied, however, with a maximum value of 0.7 μm in diameter or along the long axis. The nucleus was located in the basal part of each cell (Fig. 2). In addition to round granules which have very high elecron-density, large numbers of mitochondria, free ribosomes, fibrous structures and a small number of clear bodies were present in the cytoplasm (Figs. 2, 3 and 4). The rough-surfaced endoplasmic reticulum and Golgi apparatus were well-developed in the perikaryal cytoplasm (Figs. 6 and 7). Microridges, which were clearly revealed by scanning electron microscopy [1], were widely distributed on the free surface of the apical cells. The cell surfaces facing adjacent cells bore many cellular processes (Figs. 2, 3 and 6). Underlying cells without free surfaces showed almost the same configurations as the cells located on the free-surface side. However, the number of round granules was reduced in the cells in this underlying area. Other kinds of cells could not be found at the top of the papillae. Tight and intermediate junctions were clearly visible between the adjacent cells that were located on the free-surface side. Desmosomes were widely distributed between the adjacent cells throughout the epithelium at the top of papillae (Figs. 3 and 5).

Most of the epithelium at the base of the papillae was composed only of mucus cells and ciliated cells (Fig. 8). A large part of the cytoplasm of mucus cells was filled with mucus granules of varying degrees of electron-density. The nucleus was located in the basal part of each cell. Rough-surfaced endoplamic reticulum and Golgi apparatus were distributed mainly in the perikaryal cytoplasm of mucus cells. In some cells, microvilli on the free surface of cells were not very distinct (Fig. 9).

In ciliated cells, the nucleus was located in the basal region of each cell (Fig. 8). In the cytoplasm, mitochondria and ribosomes were widely distributed. A small number of electron-dense vesicles was seen. Basal bodies could be seen just beneath the free surface of the cells. Cilia and microvilli were located on the free surface. Tight junctions and desmosomes were intercalated on the adjacent surfaces of neighboring cells in both mucus and ciliated cells (Fig. 10).

In addition to these cells, a very small number of mitochondria-rich cells or flask cells were located within the epithelium. These cells were electron-lucent and contained many mitochondria and glycogen granules. However, they did not contain any secretory granules. Each such cell rested on the basal lamina, and its apical side reached the free surface against the oral cavity. Distinct, fine, cellular protrusions were distributed in a compact array on the free surface (Fig. 11).

In the intemediate region from the top to the base, the change in apical cells to mucus or ciliated cells was not drastic. The border between these two areas contained all types of cells, as observed under both the light and electron microscope (Figs. 1 and 2).

There were no histological differences between males and females with regard to the present results.

DISCUSSION

In mammals, almost all of the lingual epithelium is composed of stratified squamous epithelium, and various degrees of keratinization of the epithelium have been observed in some areas [6–8]. By contrast, no keratinization of any sort could be

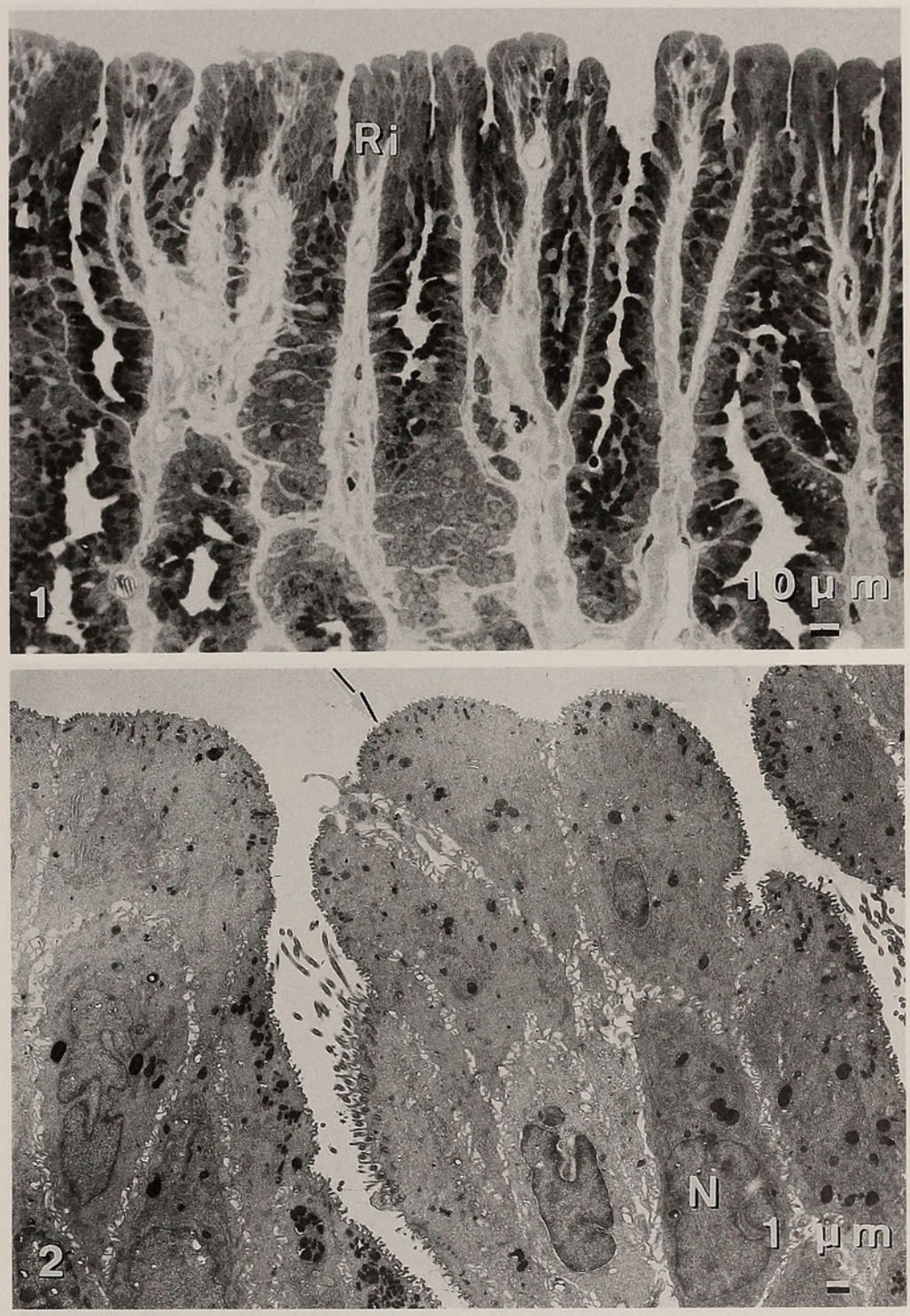


FIG. 1. Light micrograph of the lingual dorsal mucosa from a Japanese toad, *Bufo japonicus*, from Epon-Araldite embedded material. Ri: ridge-like papilla.

FIG. 2. Transmission electron micrograph of the apical cells in the epithelium from the tip of a ridge-like papilla in *Bufo japonicus*. N: nucleus. Arrow indicates microridges.

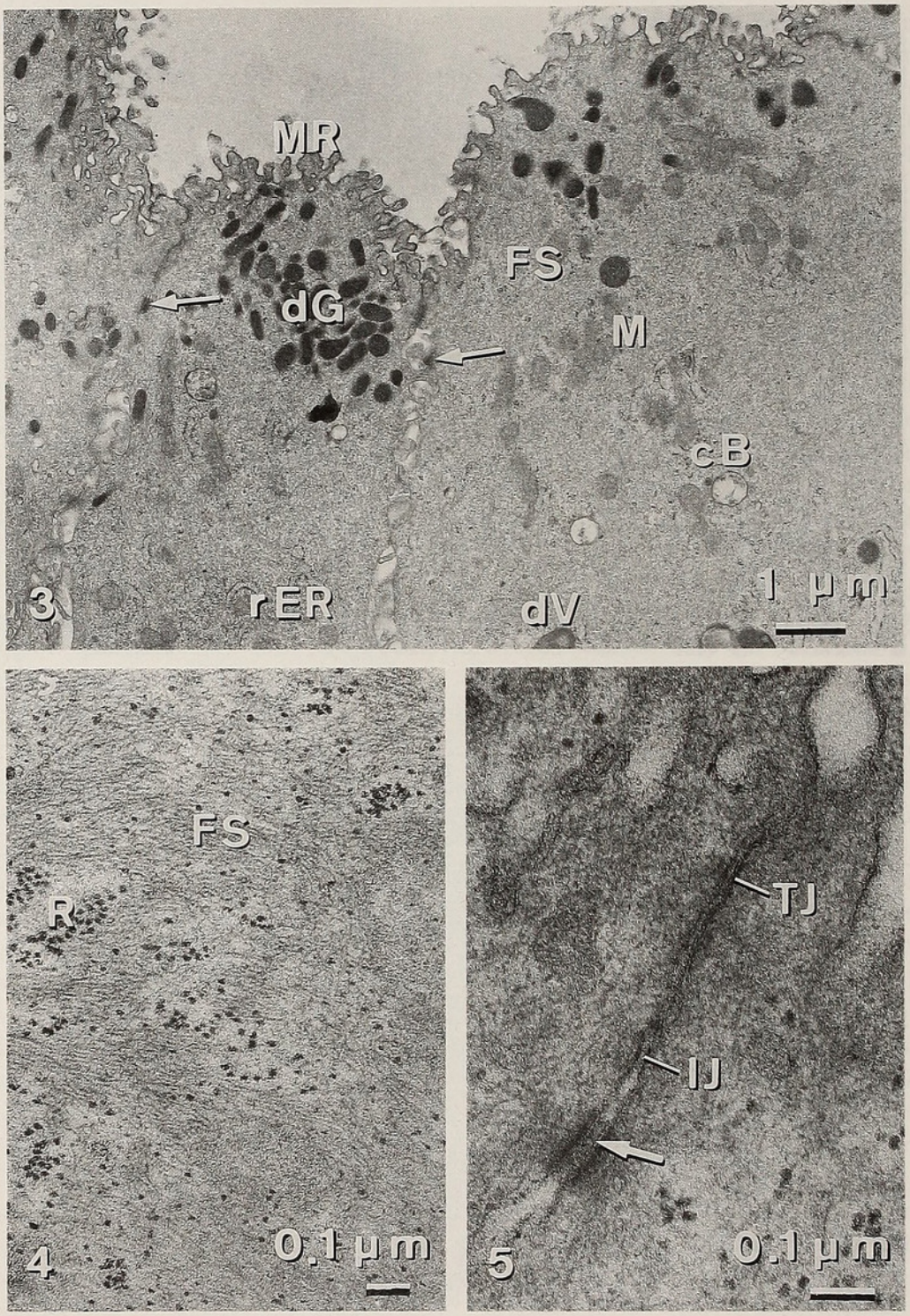
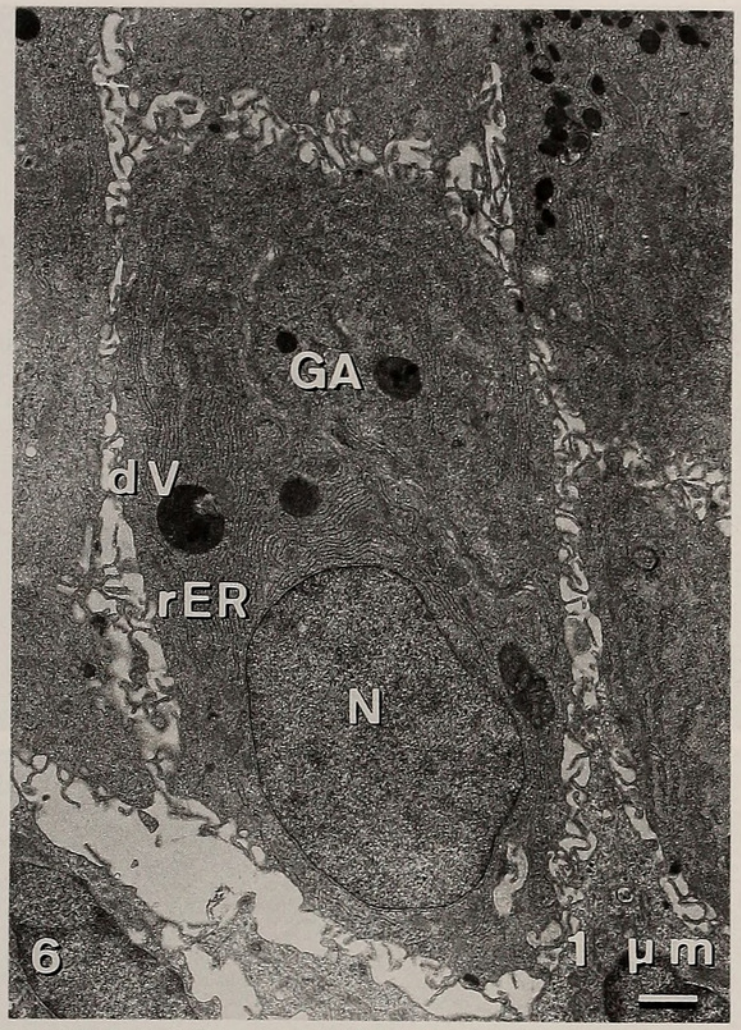


Fig. 3. Transmission electron micrograph of the epithelial cells at the tip of a ridge-like papilla. M: mitochondria, rER: rough-surfaced endoplasmic reticulum, MR: miocroridges, FS: fibrous structure, dG: electron-dense, round granules, cB: electron-lucent bodies. Arrows indicate desmosomes.

Fig. 4. Higher magnification of the cytoplasm of an apical cell. FS: fibrous structure, R: ribosomes.

Fig. 5. Higher magnification of the junctional complex of the adjacent apical cells. TJ: tight junction, IJ: intermediate junction. Arrow indicates desmosome.


GA
dV
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N
6
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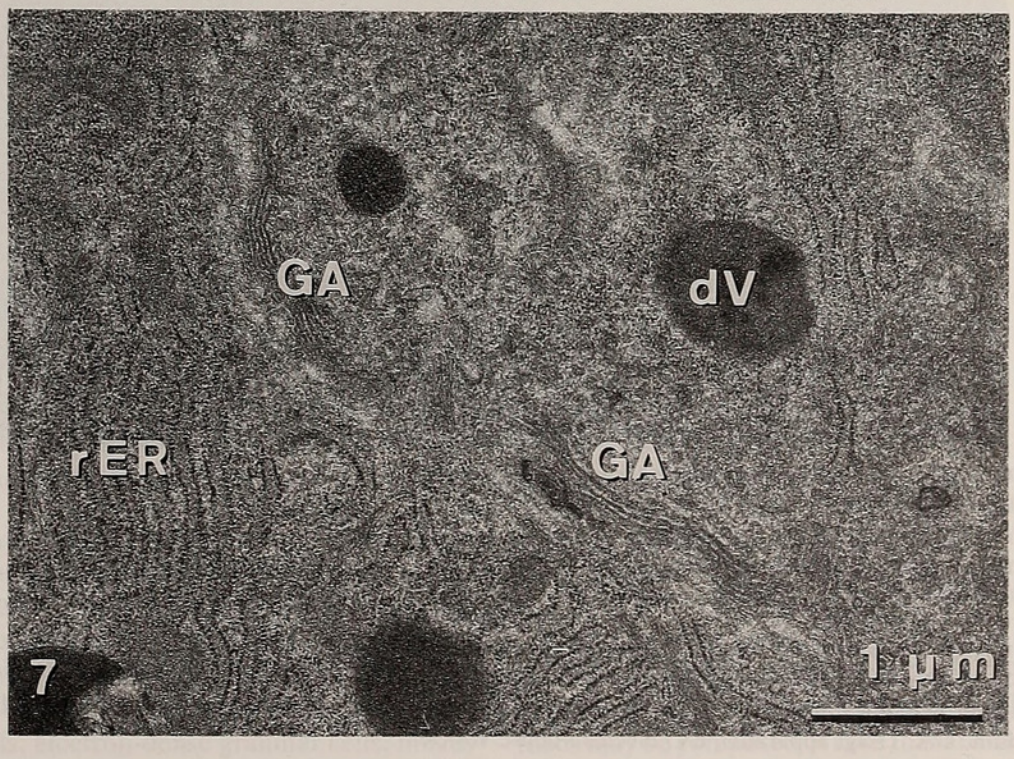
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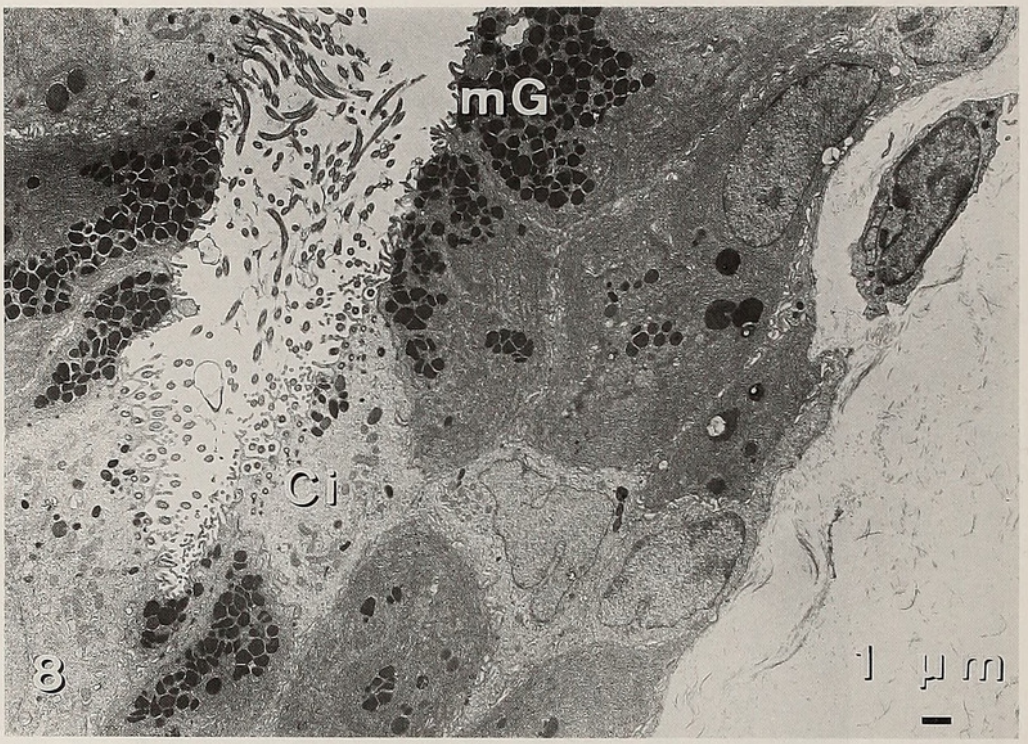

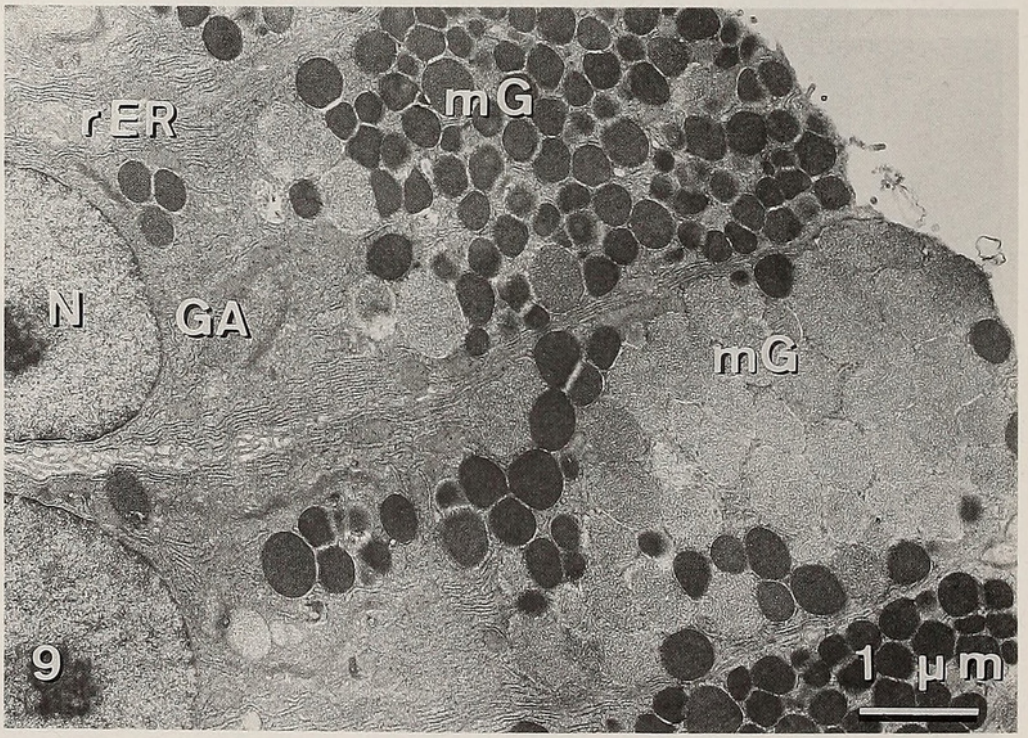

Fig. 8. Transmission electron micrograph of the epithelium of the basal part of a ridge-like papilla. mG: mucus granules, Ci: ciliated cell.

Fig. 9. Transmission electron micrograph of the mucus cells in the basal part of a ridge-like papilla. N: nucleus, rER: rough-surfaced endoplasmic reticulum, GA: Golgi apparatus, mG: mucus granules.

Fig. 6. Transmission electron micrograph of the basal area of the epithelial cells at the tip of a rigde-like papilla. N: nucleus, rER: rough-surfaced endoplasmic reticulum, GA: Golgi apparatus, dV: electron-dense vesicle.

Fig. 7. Higher magnification of the Golgi apparatus of the cell shown in Fig. 6. rER: rough-surfaced endoplasmic reticulum, GA: Golgi apparatus, dV: electron-dense vesicle.

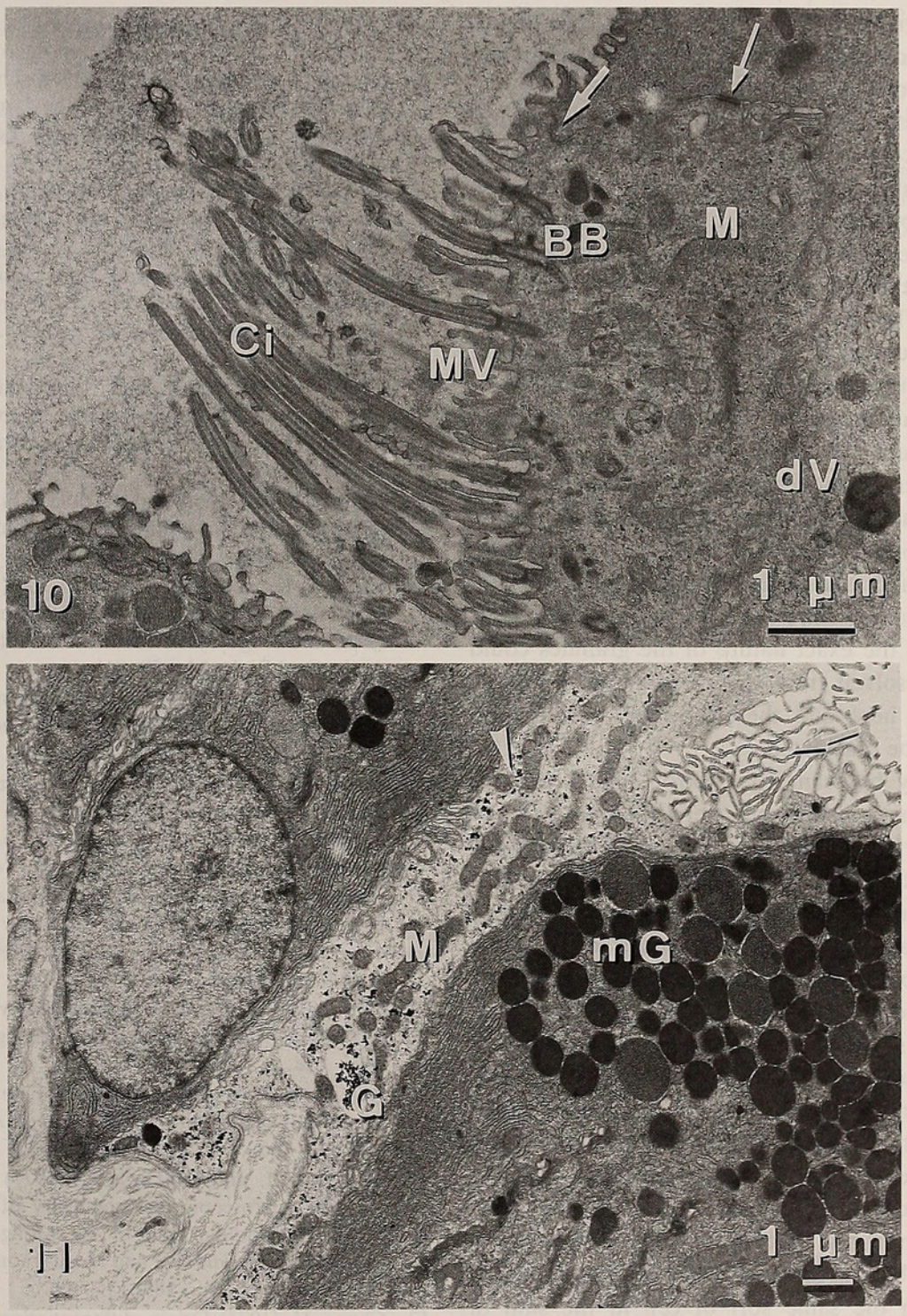

Fig. 10. Transmission electron micrograph of a ciliated cell in the basal part of a ridge-like papilla. M: mitochondria, Ci: cilia, MV: microvilli, BB: basal bodies, dV: electron-dense vesicles. Thick arrow indicates tight junction. Thin arrow indicates desmosome.

Fig. 11. Transmission electron micrograph of a mitochondia-rich cell (arrowhead) in the papillar epithelium. M: mitochondria, G: glycogen granules, mG: mucus granules. Arrow indicates long cellular protrusions.

recognized in the lingual epithelium of the toad. Instead, the epithelium was composed of several kinds of cells: electron-dense granular cells, mucus cells, ciliated cells, and other cells. These observations imply that the lingual dorsal epithelium of toads may be composed of cells that are morpholog-

ically, rather similar to the epithelial cells of the mucosa located in the gastrointestinal tract and/or the trachea of mammals.

The present study revealed that the proportion of mucus cells in the epithelium of the toad tongue was obviously larger than that in the epithelium of the frog tongue [4]. This suggests that the epithelium of the toad tongue may be more important in the secretion of mucus than that of the frog tongue. The mucus granules are variable in density. Kurosumi *et al.* [9] point out that in rat jejunal epithelium, younger granules seem to be relatively denser; this is also the case in the epithelium of toad tongue.

There are some designations that can be attributed to the electron-dense granules found in the apical papillar epithelium, such as, the immature form of mucus granules [9], serous granules [10–12], and other different types of granules [13]. However, the exact nature of the granules could not be resolved from the morphological aspects described here and therefore, this problem remains to be elucidated.

A study by scanning electron microscopy [1] showed that ciliated cells were not found on the dorsal surface of the toad tongue. This result is coincident with the present observations that ciliary cells are located exclusively in the basal area of the papillae. The pattern of distribution of ciliary cells obviously differs between the lingual epithelia of the toad and the frog [3].

A very small number of mitochondria-rich cells or flask cells were found within the epithelium. The cells may very likely be the same kind of cells found in the epidermis of amphibia [14, 15]. Significantly developed cellular protrusions such as those found on the mitochondria-rich cells of the amphibian lingual epithelium in the present study have also been recognized under conditions of high salinity in the same sort of cell in the skin of Xenopus [16]. Fox [15] surmised that the flask cells may be involved in the elimination of bicarbonate [17] and in osmoregulation [18] because of their capacity to transport ions [19]. Thus, it is possible that the mitochondria-rich cells of the amphibian lingual epithelium may also have the capacity to transport ions.

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