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FINE STRUCTURE OF THE DORSAL LINGUAL EPITHELIUM OF THE FROG, RANA RIDIBUNDA

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The structure of the dorsal lingual epithelium of the frog, Rana ridibunda was investigated by light and transmission electron microscopy. Irregular, undulant papillae were distribu-ted over the entire dorsum of the tongue. These papillae were lined by pseudostratified columnar or sometimes simple columnar epithelium. Electron microscopy revealed that, epithelium of the papillae was composed of several kinds of cells: electrondense granular cells, mucus cells, and ciliated cells. Granular cells were located over all the papillar epithelium, and these cells contained numerous electrondense granules and a few electronlucent vacuoles. In addition to these granular cells, a number of ciliated cells that contained cilia and microvilli located on their free surface, and mucus cells that contained mucus granules in almost all the cytoplasm were located within the epithelium. The purpose of this study was to clarify the cytological and histological structure of the dorsal lingual epithelium of Rana ridibunda.
Key words: *Frog, lingual epithelium, and electron microscopy*

Rana ridibunda'nın dorsaldil epitelinin ince yapısı

Bir kurbağa türü olan Rana ridibunda'nın dorsal lingual epitelinin yapısı ışık ve elektron mikroskopik olarak incelendi. Dilin bütün dorsal yüzünde düzensiz, dalgalı şekilli papillalar bulunmaktaydı. Bu papillalar yalancı çok katlı prizmatik, bazen tek katlı prizmatik epitel ile döşeliydi. Elektron mikroskopik olarak, papillar epitelin elektron yoğun granüler hücreler, mukus hücreleri, silli hücreler gibi çeşitli hücrelerden yapılı olduğu gözlendi. Papillar epitelde çok sayıda elektron yoğun granüller ve az elektron lüsent vakuoller içeren granüllü hücreler bulunmaktaydı. Epitelde bu hücrelere ilaveten, yüzeyinde siller ve mikrovilluslar içeren silli hücreler ve hemen hemen bütün sitoplazması mukus granülleri ile dolu mukus hücreleri yer almaktaydı. Çalışmamızın amacı, Rana ridibunda'nın dorsal lingual epitelinin sitolojik ve histolojik yapısını aydınlatmaktır.
Anahtar kelimeler*: Kurbağa, lingual epitel, elektron mikroskopi.*

The epithelium of the skin and oral cavity is stratified squamous in type in mammals. The upper and lower surfaces of the tongue are also covered by stratified squamous epithelium. On the upper surface the mucosa shows numerous small protuberances, called papillae. Four types of papillae are present: Filiform papillae, fungiform papillae, circumvallate papillae and foliate papillae. All papillae contain numerous sensory nerve endings for touch; taste buds are associated with all, except the filiform papillae[1-4]. The papillae are variable in relative proportion and localisation in different genera and species. Foliate papillae are rudimentary in humans but well developed in rabbits[2]. Morphological and histological features of many tissues are closely related to the environment in which the organisms live. In the present study, we investigated the gross and fine structure of the dorsal lingual epithelium of the frog, *Rana ridibunda*. *Rana ridibunda* is an amphibian and it uses its tongue while catching foods[5].

MATERIALS AND METHODS

Six frogs (three males and three females) were used in the present study. The animals were sacrificed by decapitation and the anterior parts of their tongues were excised and cut into pieces. These were fixed in 3% gluteraldehyde buffered with 0.2 M NaH2PO4+ NaHPO4 (pH=7.2-7.3), and postfixed in 0.1% osmium tetroxide buffered with 0.2 M NaH2PO4+NaHPO4 (pH=7.2-7.3). Specimens were dehydrated in acetone and embedded in Araldite CY 212. Semi thin sections were studied with toluidin blue. Ultrathin sections

were stained with uranyl acetate and lead citrate and examined in a JEOL-100SX electron microscope.

RESULTS

Many papillae were observed on the dorsal lingual surface of *Rana ridibunda* by light microscopy of semi-thin sections of Araldide embedded material. These papillae were not homologous to the typical papillae in mammals. These irregular, undulant and generally branching papillae were distributed over the entire dorsum of the tongue. The connective tissue penetrated the center of each papilla. These papillae were lined by pseudostratified columnar or sometimes simple columnar epithelium. It was observed that the epithelium of the papillae was composed of several kinds of cells: electron-dense granular cells, mucus cells and ciliated cells. We investigated fine structure of these cells in this study. We could not see the taste buds within this epithelium. The columnar cells that contained many granules densely stained by toluidin blue occupied the large part of the epithelium. Each cell rested on the basal lamina and apical portions of many of these cells reached the free surface against the oral cavity. The nuclei were located basally. Large cells, in which almost all the cytoplasm was filled with mucus, were scattered among granular cells. The mucus content of these cells was stained blue by toluidin blue. The flattened nuclei were located in the basal part of the cells. The connective tissue of the lamina propria contained many capillaries (Figures 1,2) but a few nerve fibers.

Electron microscopy revealed that, columnar granular cells contained a large number of electron-dense granules and a few electron-lucent vacuoles. The granules were observed mainly in the apical cytoplasm. Some of the granules were rounded or rod-shaped, but most granules were irregular in shape. In addition to these granules, these cells often contained a small number of electron-lucent vacuoles (Figures 3-5). Some electron-lucent vacuoles were seen just beneath the free surface of the cell. The unit membrane of these vacuoles was fused with the plasma membrane (Figure 3). The vacuoles were located adjacent to the granules (Figures 3,4). The electron lucent vacuoles were seen to contain electron-dense areas in their peripheral regions or attached to the electron-dense granules (Figure 5). A well developed rough endoplasmic reticulum; many ribosomes and mitochondria were dispersed in the cytoplasm around the electron-dense granules (Figures 3-5). Sparse microvilli were located on the free surface of some cells (Figure 3). The lateral cell surfaces facing adjacent cells harbored many cellular processes (Figures 3,4). Many desmosomes intercalated on the adjacent surfaces of neighbouring cells (Figures 4,5).

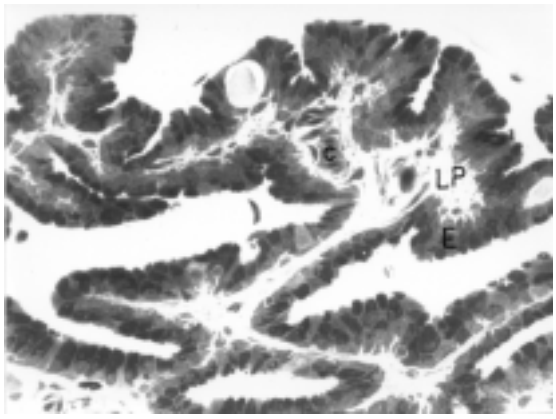

Figure 1: Irregular, undulant and generally branching papillae are distributed over the entire dorsum of the tongue. The connective tissue of lamina propria (Lp) penetrates the center of the papillae. These papillae are lined by pseudostratifed columnar or sometimes simple columnar epithelium (E). A capillary (c) containing nucleated erythrocytes is observed beneath the epithelium. Toluidin bluex20.

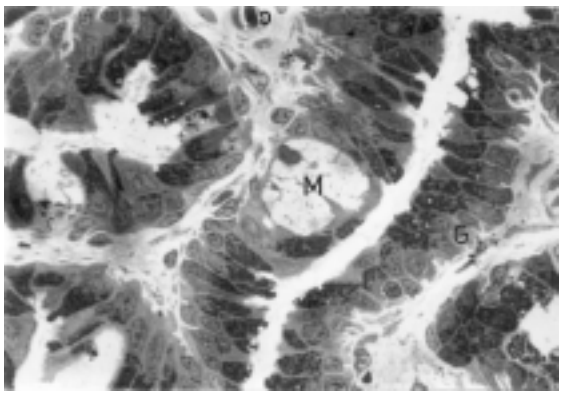

Figure 2: Electron-dense granular cells and mucus cells are observed within the epithelium The large part of the epithelium is occupied by the columnar cells that contain many granules densely stained by toluidin blue (G). Large cells, in which almost all the cytoplasm is filled with mucus, (M) are scattered among granular cells. A capillary (c) is observed in the connective tissue. Toluidin bluex40.

Columnar ciliated cells containing many kinocilia and long branching microvilli on their

exterior surfaces were scattered among the granular cells within the epithelium (Figure 3). Just beneath the free surface of the cells, basal bodies could be recognised. Cilliary rootles were distributed throughout the apical cytoplasm (Figure 6). The nucleus was sometimes irregular in shape (Figures 3,4). Mitochondria and ribosomes were widely distributed in the cytoplasm. A small number of electron-dense granules were seen especially in the apical part of the cytoplasm (Figures 4,6).

Large mucus cells, which were clearly visible by light microscopy, were located between the granular cells (Figures 1,2). A large part of the cytoplasm of mucus cells was filled with mucus granules. The nucleus was located in the basal part of each cell (Figure 7). The mucus granules were of varying degree of electron density. A well-developed rough surfaced endoplasmmic reticulum and many mitochondria were distributed mainly in the cytoplasm around the nucleus (Figures 7,8).

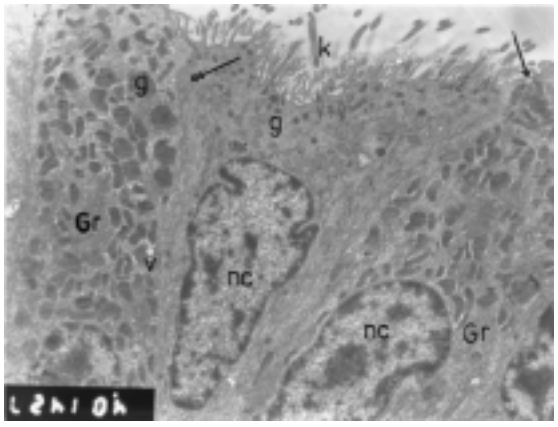

Figure 3: Granular and ciliated cells are observed. Granular cells contain a large number of electron-dense granules (g) and a few electron-lucent vacuoles (v). Nucleus (nc) is located basally. The granules are observed mainly in the apical cytoplasm. Some of them are rounded or rod-shaped, but most granules are irregular in shape. Some electron-lucent vacuoles are seen just beneath the free surface of the cell (arrow). A well-developed rough endoplasmic reticulum (Gr) is observed. The lateral cell surfaces facing adjacent cells bore many cellular processes (double arrow-head). Columnar ciliated cells containing many kinocilia (k) and long branching microvilli on their exterior surfaces are scattered among the granular cells. Mitochondria and ribosomes are widely distributed in the cytoplasm. A small number of electron-dense granules (g) is seen especially in the apical part of the cytoplasm. Uranyl acetate and lead citrate x 4.000.

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

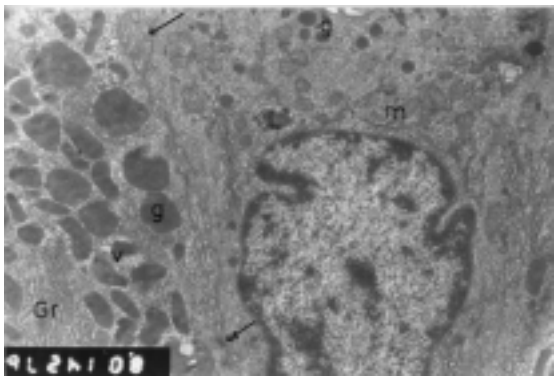

Figure 4: Granular and ciliated cells are observed. Granular cells contain a large number of electron-dense granules (g) and a few electron-lucent vacuoles (v). Some of the granules are rod-shaped, but most granules are irregular in shape. Some electron-lucent vacuoles are seen next to the granules. A well-developed rough endoplasmic reticulum (Gr) is observed. The lateral cell surfaces facing adjacent cells bore many cellular processes (arrow). A desmosome located between granular and ciliated cell is seen (double arrowhead). Mitochondria (m) and ribosomes are widely distributed in the cytoplasm. A small number of electron-dense granules (g) and lysosomes (L) are seen especially in the apical part of the cytoplasm. Uranyl acetate and lead citrate x 8.000.

DISCUSSION

The environment that the members of the genera and species live in may be different in many respects. These differences might be expected to affect the morphological and histologycal features of many tissues. Eşrefoğlu et al[6] reported some important histological differentces in the skin of the fish and frog. They observed mucus cells in the epidermis of the fish and mucous and poison glands in the dermis of the frog.

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[1-4,6,7]. By contrast, no keratinization of any sort could be recognised in the lingual epithelium of the *Rana ridibunda* in our study. Instead, the epithelium was composed of several kinds of cells: granular cells, mucus cells and ciliated cells. This observation implies that, the lingual dorsal epithelium of *Rana ridibunda* may be composed of cells that are morphologically similar to the epithelial cells of the esophagus of Rana ridibunda and trachea of mammals. Ciliated cells and mucus cells are located in the esophagus epithelium of the Rana ridibunda. Small and large intestines of Rana ridibunda

have many goblet cells as in the mammalian intestines[4,8].

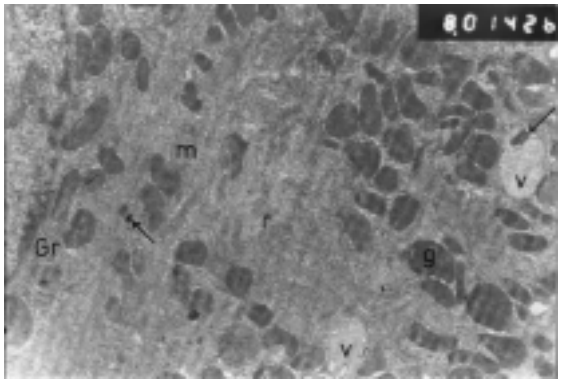

Figure 5: The apical cytoplasm of the granular cell. Many electron-dense granules (g) and a few electron-lucent vacuoles (v) are observed. Some electron-lucent vacuoles are seen just beneath the free surface of the cell. The electron lucent vacuoles are seen to contain electron-dense areas in their peripheric regions (arrow) or attached to the electron-dense granules. A well developed rough endo-plasmic reticulum (Gr), many ribosomes (r) and mitochondria (m) are dispersed in the cytoplasm around the electron-dense granules. A desmosome is seen on the adjacent surfaces of neighbouring cells (double arrowhead). Uranyl acetate and lead citrate x 8.000.

Papillae are composed of epithelium and connective tissue of lamina propria[2]. In the present study we observed irregular, undulant and generally branching papillae over the entire dorsum of the tongue. These were morphologically different from that of the mammals. Similar papillae have been observed in *Rana rugosa* and *Rana catesbeiana*. But these papillae were described as filiform or fungiform papillae[9,10]. In the present study we observed that the epithelium of the papillae was pseudostratified columnar or simple columnar in type. We could not see the taste buds within this epithelium. The sense of taste in frogs involves papillary and nonpapillary receptors. The first are located on the apex of the fungiform papillae on the dorsal surface of the tongue. Nonpapillary receptors are located on the floor of the mouth and on the palate[11]. The sensorial area or sensory disc including mucous, supporting and neuroepithelial cells is located on the freetop of the fungiform papilla of *Rana rugosa, catesbeiana*, *nigromaculata, esculanta* and *Bufo japonicus*. This area is homologous to the taste buds of mammals[11,12]. There are some comperative studies on some species of Rana and Bufo in order to establish the extent of similarities and differences of the taste cells. The structures related to the taste sense have some morphologically and histologically different features[12,13]. In frogs, sensorial area is separated from the lateral wall of the fungiform papilla by ciliated cells[11]. In *Rana*, ciliated cells are seen on the surface of the filiform papillae and in the area surrounding the sensory disc[9]. We could not observe the sensorial area in the dorsal lingual epithelium in *Rana ridibunda*. The sensorial area is richly innervated by nerves. Taste buds and sensorial discs receive a number of sensory fibers. A sensory nerve fiber not only branches before entering the papilla but it also branches close to the sensory disc where its terminal branches contact more than one sensory cell[11]. We observed rare nerve fibers and synapses within the epithelium. These may be involved in the mechanoreceptor function.

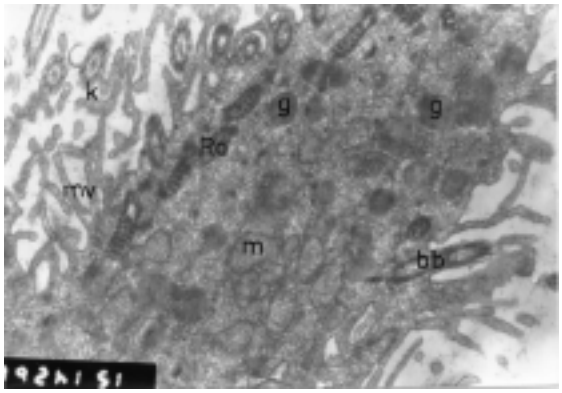

Figure 6: Apical cytoplasm of the ciliated cell. Columnar ciliated cells contain many kinocilia (k) and long branching microvilli (mv) on their exterior surfaces. Just beneath the free surface of the cells, basal bodies (bb) can be recognised. Cilliary rootless (Ro) are distributed throughout the apical cytoplasm. Mitochondria (m) and ribosomes are widely distributed in the cytoplasm. A small number of electron-dense granules (g) are seen especially in the apical part of the cytoplasm. Uranyl acetate and lead citrate x 15.000

Granular, mucus and ciliated cells have been observed in some genera and species of the frog[7,9,10,13]. The electron-dense granules located in the cytoplasm of the granular cells may be analogous to serous granules found in the salivary glands of mammals, but may be mucous or other types of granules[9,10,13]. There are two possible explanations for the presence of electron-dense granules in the whole papillar epithelium: one is that they may be serous or may be immature forms of the mucus granules[9,13]. The mucus granules are variable in density; younger granules are relatively

denser[7]. We observed mucus granules of varying degrees of electron density. But we think that the electron-dense granules are unrelated to the mucus granules because we found no evidence of any transitional stages existing between the electron-dense granules and the mucus granules in the granular cells. Additionally, morphology of granular cells and mucus cells were different. Thus, we suggest that these granules are serous granules. The granules in Rana esculanta contain both mucous substance and protein[13].

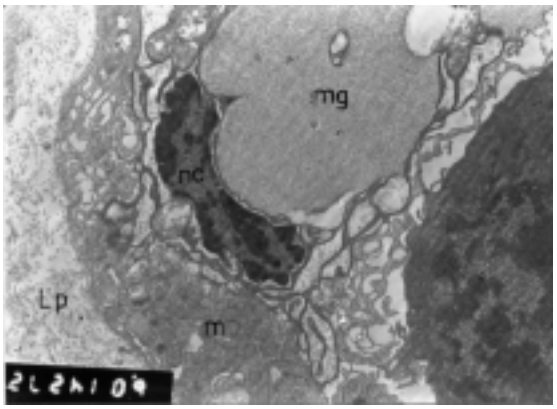

Figure 7: A large part of the cytoplasm of mucus cells is filled with mucus granules (mg) of varying degree of electron density. The nucleus (nc) is located in the basal part of the cell. Many mitochondria (m) are distributed mainly in the cytoplasm around the nucleus. Lamina propria (Lp) is observed beneath the basal membrane. Uranyl acetate and lead citrate x 6.000.

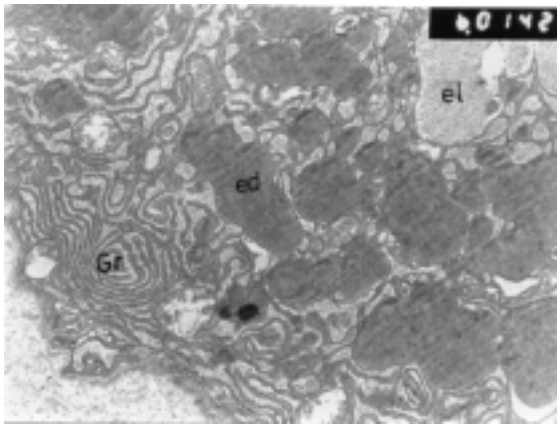

Figure 8: A large part of the cytoplasm of mucus cells is filled with mucus granules of varying degree of electron density, electron-dense (ed) and electron-lucent (el) E well-developed rough endoplasmic reticulum (Gr) is observed in the basal part of the cell. Uranyl acetate and lead citrate x 6.000.

It is known that the serous glands of mammals secrete or discharge contents of serous granules through exocytosis. The membrane of the granule fuses with the plasma membrane at the lumen of intercellular canaliculus, with subsequent openings of the region formed by the fused membranes. Direct discharge of the contents of electron-dense granules into the oral cavity has not been observed in *Rana rugosa* and *Rana catesbeiana*[9,10,13]. It is possible that after some electron-dense granules have disagregated to form an electron-lucent vacuole; the contents of the electron-lucent vacuole are discharged into the oral cavity by exocytosis. In Rana cancrivora, granules are discharged in an electron-dense or electron-lucent form[13]. In our study we observed many electron-dense granules just beneath the plasma membrane. There were some electron-lucent vacuoles next to the granules. Some vacuoles were seen to contain electron-dense areas in their peripheral regions or attached to the electron-dense granules. The electron-dense granules may be secreted directly into the oral cavity in an unchanged form, or as granules with mixed electron-dense and lucent areas. Instead, it seems likely that after some electron-dense granules aggregate to form an electron-lucent vacuole, 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 similar to the rapid secretion of the contents of serous granules in the salivary glands of mammals, as it occurs after stimulation with pharmacological agents[10].

We think that the mucus cells are equivalent to the Goblet cells located in the gastro-intestinal tract and respiratory tract. These cells are observed in the dorsal lingual epithelium of *Rana catesbeiana, nigromaculata, esculanta*[9-11] and in *Bufo japonicus*[6]. A thick mucus layer covers the surface of the tongue in frogs[12]. This is secreted by the mucus cells. We suggest that mucus makes an unpermeable barrier against the water and facilitates the catching of insects.

Mucus, secreted from mucus cells is removed from the surface of the epithelium with the active cilliary movements of the kinocilia of the ciliated cells located adjacent to the mucus cells[1,2]. We observed ciliated cells containing many kinocilia on their external surface adjacent to the mucus cells. The ring of the

ciliated cells surrounding the sensorial area shows very active ciliary movement in frogs. Thus it is able to clean the sensorial area in order to avoid a papillar sensorial inactivation, for example to a mucous substance[11,12].

To the best of our knowledge, this is the first ultrastructural study about the dorsal lingual epithelium of *Rana ridibunda*. The light and ultrastructural features of the epithelium of the tongue in frogs are highly different from those of the mammals in many respects.

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