Morphological Features of the Salivary Glands in the Gray Short-Tailed Opossum (*Monodelphis domestica*)

（ハイイロジネズミオポッサムにおける唾液腺の形態学的特徴）

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1. Abstract

Introduction

The salivary glands produce saliva, which helps keep the mouth and other parts of the digestive system moist. Mammals normally have major and minor salivary glands. Genome of the gray short-tailed opossum was sequenced and published in May 2007. This animal has several characters that make it an important research model for mammalian systems.

The aim of this study was to reveal the morphological features of the parotid, submandibular, and posterior lingual, glands in the gray short-tailed opossum, and to compart these finding to those of other mammalian including marsupial superorder. This study revealed the histological feature of the parotid glands, the submandibular glands and the posterior lingual glands in the short-tailed opossum using lighting microscopy (LM) and the ultrastructure of the parotid and submandibular glands in the gray short-tailed opossum using transmission electron microscopy (TEM).

Materials and Methods

Materials

Samples of the parotid and submandibular glands were obtained from three gray short-tailed opossums (*Monodelphis domestica*) (Adult female, 24 months old).

Methods

LM: All specimens were fixed in 10% neutral formalin solution, and paraffin sections were made by usual methods. They were stained with hematoxylin and eosin, PAS-alcian blue pH 2.5 and mucicarmine.

TEM: There parotid and submandibular glands were sliced into small pieces, were fixed with 2% glutaraldehyde and 1% osmic acid, and then embedded in epoxy resin. Ultrathin sections were made and stained with uranyl acetate-lead citrate following the routine procedures, and were observed and photographed in a JEM-1010 (JEOL, Japan).

Results

LM: Parotid glands were composed of many lobules, and parotid acinar cells consisted only of serous secretory cells. The striated ducts revealing basal striations and intercalated ducts were also observed. The serous acinar cells have a spherical nucleus locating at the center or the base of the cells. These serous acinar cells had basophilic cytoplasm and numerous secretory granules. Their cytoplasm was partly stained with PAS.

The submandibular glands were located at the posterior and inferior portion of the tongue in the anterior neck space. The submandibular glands were composed of many lobules separated by thin fibrous septa. The submandibular glands consisted of serous and mucous acinar cells, showing mixed glands. Serous acinar cells had basophilic cytoplasm and numerous secretory granules and

their cytoplasm was partly stained with PAS. Mucous acinar cells had pale-staining cytoplasm stained with alcian blue and/or PAS and mucicarmine.

The Weber's glands were located from the filiform papillae to the back of the circumvallate papilla, and they consisted of seromucous secretory cells showing mucous-rich mixed glands. These mucous acinar cells were stained with alcian blue and/or PAS. The von Ebner's glands were located from the fungiform papillae to the around of the circumvallate papillae, and they were typically PAS positive serous acinar glands.

TEM: The parotid glands consisted only of the serous acinar cells. These secretory granules showed homogenous high electron density. The submandibular glands composed both of serous and mucous acinar cells, showing mixed glands. And, the submandibular glands possessed the special serous acinar cells. The secretory granules were divided into homogeneous density, two biphasic and annual ring types. The properties of the granule were different in each cell. However, there were some cells that every type of granules were mixed. It was suggested that the morphological variety of granules in the special serous acinar cells reflected the formation stages in the cell cycle. Striated ducts of the submandibular glands were composed of columnar epithelial cells with or without basal infoldings, although those of the parotid glands were consisted of typical columnar epithelial cells with the infoldings.

Conclusions

The gray short-tailed opossum showed morphological accordance and difference of the salivary glands among mammals, included in differences of ontogenetic feature and phylogenetic position

Keywords: salivary gland, morphology, the gray short-tailed opossum, *Monodelphis domestica*

2. Introduction

Saliva was crucial for the maintenance of oral health in that it contained a complex mixture of molecules possessing a multitude of functions, such as lubrication of the oral mucosa, defense from infections, prevention of tooth demineralization and promotion of tooth remineralization (1). Mammals normally had three large salivary glands that include the parotid, submandibular and sublingual glands (2). These major salivary glands were made from many lobular structures (3). It was told that the parotid, sublingual and submandibular glands of mammals produce serous, mucous and mixed secretions, respectively (2). The three major salivary glands collectively supplied approximately 90% of total salivary flow (4). In addition to these major salivary glands, a large number of minor salivary glands also develop and exist in the mouth (5). The posterior area of the mammalian tongue includes two sets of posterior lingual glands (6). One of the posterior lingual glands, well known as von Ebner's glands, is a group of tubuloacinar serous glands located beneath the circumvallate and foliate papillae of the tongue and their ducts open into the through at the base of the papillae (7). On the other hand, another of the posterior lingual glands, sometimes known as Weber's glands, is located on the lateral margin at the level of the foliate papillae and in the root of the tongue behind the circumvallate papillae, and their ducts of Weber's glands open into the crypts of lingual tonsils (8).

Marsupials were mammals whose young were born in a much undeveloped stage and attached to the mother's nipples, usually in a pouch (9). Despite, these jaw elements to suckle and digestive system must develop early (10). Therefore, it was considered that the structure of the salivary gland on a marsupial may have some difference in comparison with other mammals. There have been many morphological studies of major salivary glands of mammals such as human (2, 11), mouse (12- 14), and rat (2, 4, 15). And, there have been some histological reports on the posterior lingual glands of humans (16- 18), monkeys (19) and rats (20, 21). But, some of these have researched microstructures in transmission electron microscopy (TEM). Therefore we observed a salivary gland of the gray short-tailed opossum (*Monodelphis domestica*) using an optical microscope and TEM in this study and conducted histologic examination. There were some reports of the sublingual glands in human (2, 3, 8) but, there were few reports of the sublingual glands in other mammals. Therefore, it was insignificant to study the sublingual glands of opossum. The aim of this study was to reveal the histological features of the parotid, submandibular and posterior lingual glands in the gray short-tailed opossum.

3. Materials and Methods

3-1. Materials

The parotid glands, the submandibular glands and the posterior lingual glands were obtained from three gray short-tailed opossums (*Monodelphis domestica*) (female, 24 months old). The

experimental protocol was approved by Nihon University Animal Care and Use Committee (Nos.AP09MD023, AP12MD015).

3-2. Methods

1) Light Microscopic (LM) finding

Following fixation with 10% neutral formalin, the specimen was cut into several pieces and routine paraffin sections of sagittal direction at the midline and cross direction at other areas were made. The sections were stained with hematoxylin and eosin (HE), combine periodic acid-Schiff reaction (PAS)-alcian blue (AB) at pH 2.5 and mucicarmine by the usual methods.

2) Transmission Electron Microscopic (TEM) finding

The parotid glands and the submandibular glands were sliced into small pieces, and they were fixed with 2% glutaraldehyde and 1% osmic acid, and then embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate-lead citrate following the routine procedures, and were observed and photographed in a JEM-1010 (JEOL, Japan).

4. Results

4-1. Gross and LM findings

1) The parotid gland

Gross finding

In macroscopic observation, parotid glands were located at the anterior inferior ear and were flat triangle form. Their surfaces were smooth and dark reddish-brown. The size of the parotid gland was 17 x 8 x 4 mm (Fig. 1a).

LM finding

The parotid glands composed of many lobules, and parotid acinar cells consisted only of serous secretory cells (Figs. 1b, 1c). The striated ducts revealing basal striations and intercalated ducts were also observed. The serous acinar cells have a spherical nucleus locating at the center or the base of the cells (Fig. 1c). These serous acinar cells had basophilic cytoplasm and numerous secretory granules (Fig. 1c). Their cytoplasm was partly stained with PAS (Fig. 1d).

2) The submandibular gland

Gross finding

Macroscopically, bilateral submandibular glands were located in the anterior neck spaces. In the specimen with the tongue and the floor of mouse, the submandibular glands were recognized at the posterior and inferior portion of the tongue. The size of the submandibular gland was 10 x 13 x 3 mm (Fig. 2a).

LM finding

The submandibular glands composed of many lobules which were separated by thin fibrous septa (Fig. 2b). The submandibular glands consisted of seromucous secretory endpieces and the

submandibular glands were mixed glands (Figs. 2b, 2c). The myoepithelial cells having spindle shape were often recognized around the secretory endpieces. The striated ducts revealing basal striations and intercalated ducts were also observed. The serous acinar cells have a spherical nucleus locating at the center or the base of the cells (Fig. 2c). These serous acinar cells have basophilic cytoplasm and numerous secretory granules (Fig. 2c). Their cytoplasm was partly stained with PAS (Fig. 2d). The mucous acinar cells have flattened or oval nucleus mostly located at the base of the cells (Fig. 2c). Their cytoplasm was pale-staining with hematoxylin, and stained with alcian blue and/or PAS and mucicarmine (Figs. 2c-2e). Some ducts and microvessels were also recognized in the lobules (Figs. 2c-2e).

3) The posterior lingual gland

Gross finding

Macroscopically, the size of the tongue was 30 x 8 x 10 mm (The size of width and thickness was that of the base of the tongue) (Fig. 3a).

LM finding

The posterior lingual glands consisted of two sets of the minor salivary glands (Fig. 3b). One of the posterior lingual glands was named Weber's glands. These Weber's glands were located from the filiform papillae to the back of the circumvallate papilla and mainly existed in the upper and middle muscular layer, and they were the tubuloacinar glands consisting of seromucous secretory cells and were mucous-rich mixed glands (Fig. 3c). These mucous acinar cells were composed of two kinds of cells, and one has basophilic cytoplasm but another hardly showed basophilia. Another of the posterior lingual glands was called von Ebner's glands. These von Ebner's glands were located from the fungiform papillae to the around of the circumvallate papillae and mainly existed in the upper muscular layer, and they were typically serous glands (Fig. 3d). Both of the mucous acinar cells were columnar, and their nucleus was flattened and located at the base of the cells (Fig. 3c). Their cytoplasm was pale-staining with hematoxylin and stained with alcian blue and/or PAS (Fig. 3e). The serous acinar cells were pyramidal in shape, and typically have a spherical nucleus, basophilic cytoplasm and numerous apically-located secretory granules (Figs. 1c, 1d). Their cytoplasm was partly stained with PAS (Fig. 3e).

4-2. TEM finding

1) The parotid gland

The acinar cells were pyramidal, and these nuclei existed at the basal side of the cells (Fig. 4a). A number of mitochondria and tubular or vesicular ER (endoplasmic reticulum) existed throughout the acinar cells. The secretory granules were observed in the lumen side (Figs. 4a, 4b). The myoepithelial cells were flat, and existed along the basement membrane (Fig. 4c). The secretory granules were homogeneous high electron density (Fig. 4d). The striated ducts consisted of columnar cells. The striated epithelium had basal infoldings associated with mitochondria (Fig. 4e). The

intercalated ducts were short segments lined by cuboidal cells (Fig. 4f).

2) The submandibular gland

The secretory units were composed both of serous and mucous acinar cells, showing mixed glands. An irregularly shaped nucleus was found in distal pant of acinar (Fig. 5a). A number of mitochondria and tubular or vesicular ER existed throughout the acinar cells. The secretory granules were observed in the lumen side (Figs. 5a, 5b). The myoepithelial cells were flat, and contain abundant myofilaments (Fig. 5c). There were four different types of the secretory granule in serous acinar cells: i) homogeneous high electron density type, and biphasic granule type with ii) high electron dense core, iii) low electron dense core, and iv) annual ring type (Fig. 5d). Some serous acinar cells had several types of granule (Fig. 5e). The secretory granules of mucous acinar cells showed homogeneous low electron density (Fig. 5f). Striated ducts of submandibular glands include both epithelium cells with and without basal infoldings, although typical striated ducts are shown in parotid glands (Figs. 5g, 5h). The Intercalated ducts were short segments lined by cuboidal cells (Fig. 5i).

5. Discussion

The spherical secretory end pieces of parotid glands were all serous (3). The acinus was composed of serous secretory cells that contain many secretory granules having an electron-lucent profile and are situated in the supranuclear cytoplasm (2). There have been some researches on the histological architecture of the parotid glands of mammals such as human (2, 3, 8), mouse (12) and rat (2, 4). Amano et al. (2) reported that both human and rodent parotid glands were composed of pure serous acini. The human parotid gland was well characterized intralobular adipose tissues, whereas the adipocytes are not prominent in the rodent parotid gland. The parotid gland consisted only of serous acinar cells without regard to food habit and phyletic lineage.

It is well-known that the submandibular glands of human contain serous end pieces and mucous capped with serous demilunes; thus they are mixed glands (3). Furthermore, the proportions of serous and mucous secretory end pieces in the human salivary glands may vary from lobule to lobule and among individual glands, serous acinar cells significantly outnumber the mucous acinar cell; thus the human submandibular glands are serous-dominant mixed glands (3). There have been many researches on the histological architecture of the submandibular glands of mammals for example: bat (22) as insectivore, cat (23) and dog (24) as carnivorous, mouse (13, 25, 26), rat (2, 27-32), hamster (33, 34), ferret (35), rabbit (36, 37), pig (38, 39), goat (40) and bovine (41) as herbivorous, human (2, 3, 8, 11) and monkey (42) as omnivorous in placentalia, and opossum (43-45) as insectivore, koala (46, 47) and wallaby (48) as herbivorous in marsupial. In these mammals, the secretory units of the submandibular glands except for all papers of koala (46, 47) and one report of rodents (2) consist of serous and mucous acinar cells, revealing the submandibular gland of almost of mammals are mixed

gland. In these reports, it is told that the submandibular glands of koala and rodent were composed only of serous acinar cells (2, 46, 47). In other kinds of opossum, Leeson et al. (43) and Pinkstaff (44) told that North American opossums (*Didelphis virginiana*) have the submandibular glands that revealed histological architecture of mixed glands. Furthermore, Blood et al. (45) reported that the submandibular glands of the Australian brush-tailed opossum (*Trichosurus vulpecula*) consisted of serous and seromucous acinar cells, showing mixed glands.

Histochemically, it was well-known that the epithelial mucous showed positive reaction to mucicarmine. Furthermore, it was reported that the serous acinar cells revealed PAS positive reaction, and the mucous acinar cells showed alcian blue pH 2.5 positive and/or their mixture with PAS. In the research 2, the serous and mucous acinar cells showed same reaction to PAS-AB pH2.5 staining, showing the serous acinar cells had PAS positive material corresponding to glycogen and the mucous acinar cells contained alcian blue pH 2.5 positive and/or their mixture with PAS corresponding to neutral and acid mucopolysaccarides. The submandibular glands in the gray short-tailed opossums were mixed glands composed of the serous and mucous secretory acinar cells, morphologically and histochemically.

The posterior lingual glands, one of the minor salivary glands, are divided into two groups based on property and function. It is told that von Ebner's glands of the human are located beneath the circumvallate papillae and are serous, but Weber's glands of the human are located on the lateral margin and in the root of the tongue and are purely mucous in character (8). It is summarized previous papers related and described that human von Ebner's glands are serous and human Weber's glands are mucous and seromucous (18). On the other hand, Field et al. (21) described that rat von Ebner's glands are also serous. Nagato et al. (20) reported that rat Weber's glands are mixed glands, consisting of mucous tubules that often are capped by serous demilunes. The posterior lingual glands were composed of two sets of minor salivary glands and one was von Ebner's glands and another was Weber's glands as reported previously (16, 19, 20). von Ebner's glands showed typical serous in this study and this finding was as same as others (16, 19, 20). The research 1 revealed that Weber's glands were mucous-rich mixed glands. Yuzawa et al. (16) told that human Weber's glands were mucous glands. Furthermore, Suzuki et al. (19) revealed that monkey Weber's glands were also mucous glands. On the other hand, Nagato et al. (20) reported that rat Weber's glands were mixed glands. Thus, the histological features of Weber's glands on the gray short-tailed opossum are basically in accordance with rat previous report (20).

For TEM, the parotid glands were consisted only of serous acinar cells. The submandibular glands were composed both of serous and mucous acinar cells, showing mixed glands. Striated ducts of submandibular glands were composed of columnar epithelial cells with or without basal infoldings, although those of the parotid glands were consisted of typical columnar epithelial cells with the infoldings. Shackleford et al. reported that there is resemblance in rodent submandibular

glands known as granular tubules in which intercalated duct terminate (42). According to the researches by Wilborn et al. (49) submandibular secretory units of North American opossum (*Didelphis virginiana*) had the special serous acinar cells, which differed from typical serous acinar cells. Shackleford et al. (42) reported that special serous acinar cells, which had little or no detectable mucosubstance, differ structurally from other serous acinar cells. Special serous acinar cells, as a group, appear more heterogeneous than did serous, mucous, or seromucous acinar cell types. The irregular shaped nucleus was characteristic of opossum special serous acinar cells. Wilborn et al. (49) reported that, the most notable difference was that special serous acinar cells did not possess the closely packed lamellae of endoplasmic reticulum as did the serous acinar cells described by others. Less striking differences include fewer basal infoldings and more mitochondria in special serous acinar cells than reported in the serous acinar cells of human submandibular glands. Special serous acinar cells occurred among mucous acinar cells, extend from the ends of some tubules which constituted the lumen of acinar, or encircle other tubules as demilunes. Thus, apices of some special serous acinar cells didn't reach the mucous tubule lumen. Spaces were occasionally seen between cells which possibly serve as avenues for secretion of the peripherally located special serous acinar cells to reach the lumen of the mucous acinar. Shackleford et al. (42) reported that special serous acinar cells were present in dog sublingual glands and ruminant (cow and sheep) parotid glands. Marsupialia and placentalia diverged from a mammalian common ancestor in the late Cretaceous (50). Opossum has been recognized as primitive lineage by some features including dentition (51). Special serous acinar cell seems vestigial character in mammals, because they also remain in some placental (dog, cow etc.) without regard to food habit.

Blood et al. (45) reported that the secretory granule in acinar cells of Australian brush-tail opossum (*Trichosurus vulpecula*) had filamentous material and two types of denser inclusions. Ichikawa et al. (52) reported that the granules in serous acinar cells of the monkey submandibular glands had central dense spherule and a rim of lower dense matrix. The granules in serous acinar cell of the Mongolian gerbil salivary glands had high dense of cores and bipartite structure. Takada et al. (53) reported that the granules in mucous acinar cell of the mouse parotid glands in the early postnatal had low electron density and bipartite structure. Wilborn et al. (49) reported that the cell base lacks the infoldings observed in light cells of striated duct. Two types of basal cells of striated duct contain numerous filaments and lack basal infoldings. Tamarin et al. (54) reported that dark cells and basal cells of striated duct have been observed in excretory ducts of rat submandibular glands. Results of this study demonstrated the submandibular glands of the gray short-tailed opossum possess the special serous acinar cells. Secretory granules were divided into homogeneous density type, two biphasic types and annual ring types. The properties of the granules were different in each cell. However, there were some cells that every type of granules were mixed. It seems that variety of granules in the special serous acinar cells represent stages in the cell differentiation not the

property of the cell.

In this study, the parotid glands were consisted only of serous acinar cells. The submandibular glands and the Weber's glands were composed both of serous and mucous acinar cells, showing mixed glands. The von Ebner's glands were typically serous glands. In conclusion, the special serous cells of submandibular gland were thought to be the features in Marsupials without regard to food habit .The gray short-tailed opossum shows morphological accordance and difference of the salivary glands among mammals, in concerning with marsupial phylogenetic position.

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8. Figure Legends

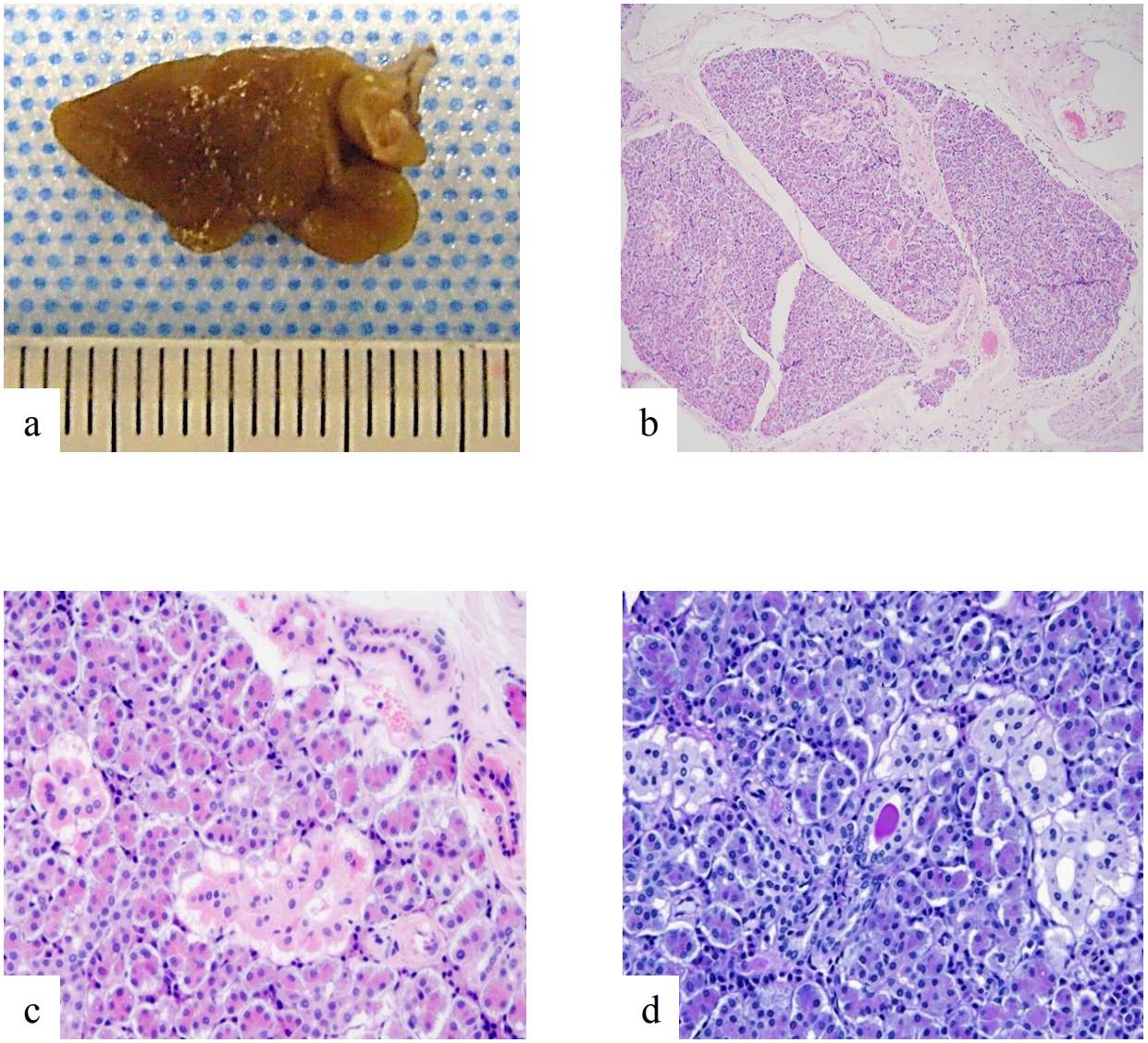
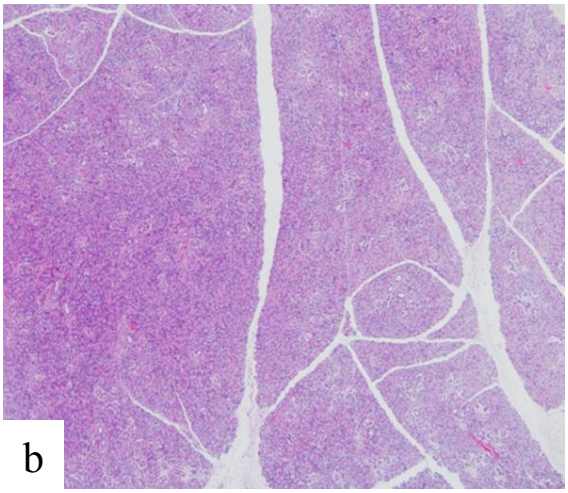


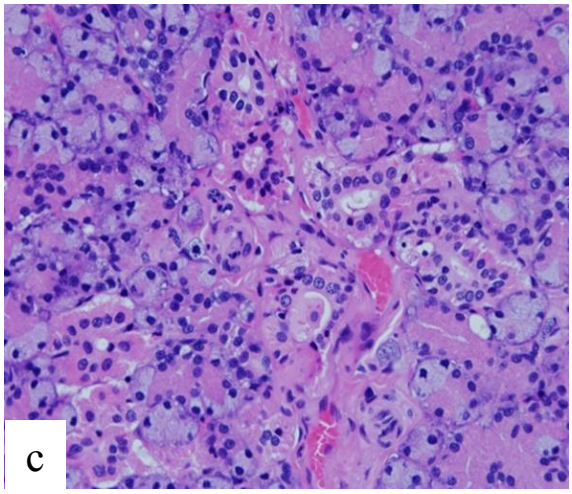
Figure 1　Gross and histological findings of the parotid gland

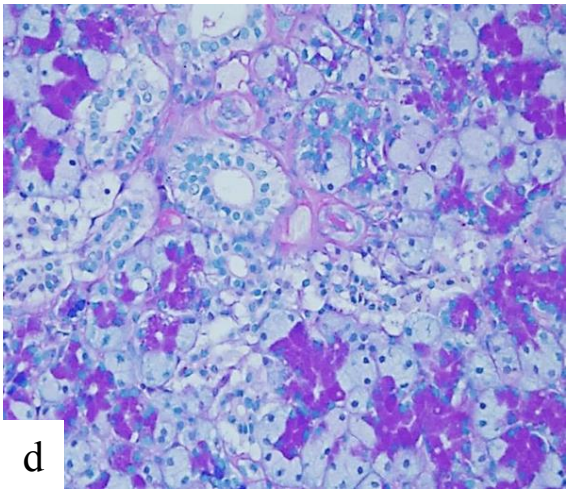
Macroscopically, the parotid glands are located at the anterior inferior ear, and are flat triangle form. Their surfaces are smooth and dark reddish-brown (a).

Microscopically, the parotid glands are composed of many lobules, and parotid acinar cells consist only of serous secretory cells (b, c). The striated ducts revealing basal striations and intercalated ducts are also observed. The serous acinar cells have a spherical nucleus locating at the center or the base of the cells (c). These serous acinar cells have basophilic cytoplasm and numerous secretory granules (d). Their cytoplasm is partly stained with PAS (d).

a: gross, b: HE x40, c: HE x400, d: PAS-Ab x400







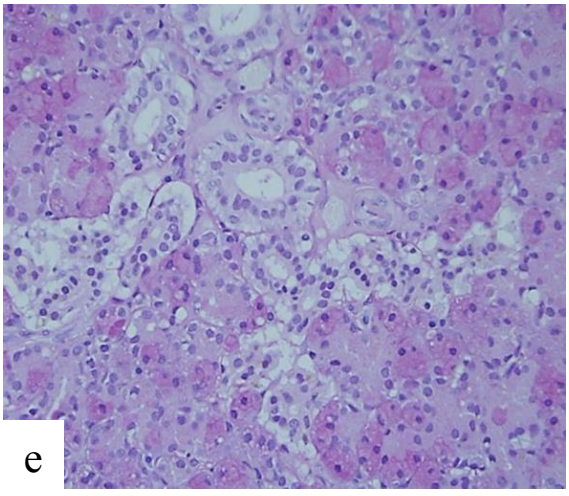
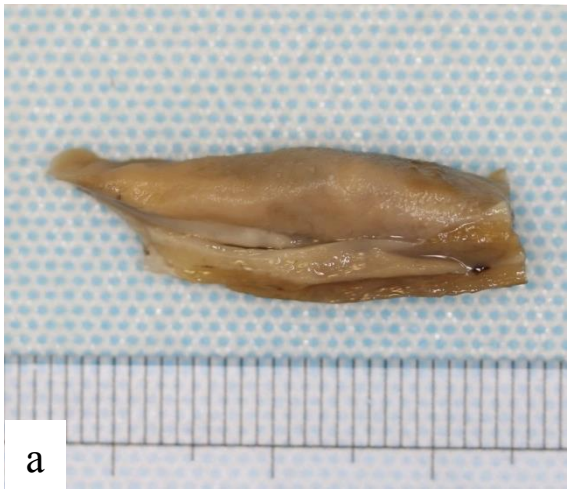


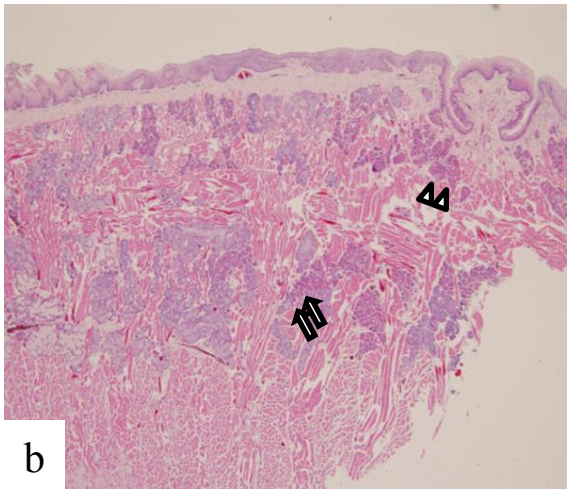
Figure 2 Gross and histological findings of the submandibular gland

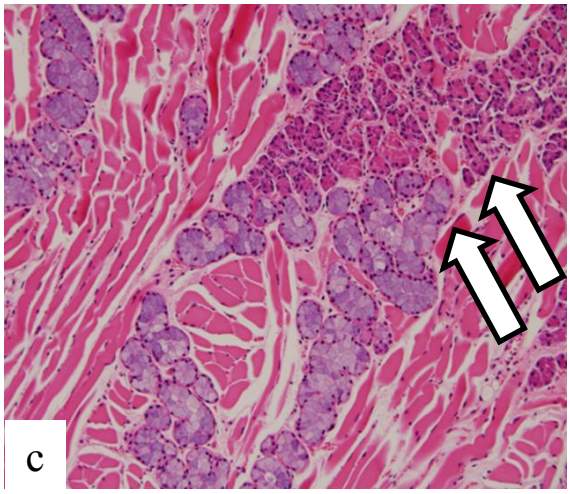
Macroscopically, the submandibular glands (circle) are located in the anterior neck spaces, are recognized at the posterior and inferior portion of the tongue (a).

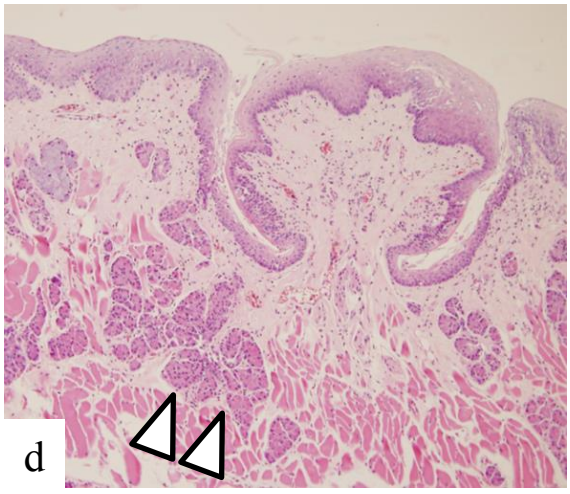
Microscopically, the submandibular glands are composed of many lobular architectures (b).
The submandibular glands are consisted of the seromucous acinar cells, and are mixed glands (c).
The mucous acinar cells are stained with alcian blue and/or PAS, and serous acinar cells are partly stained with PAS (d). Only mucous cells are stained with Mucicarmine (e).

a: gross, b: HE x40, c: HE x400, d: PAS-Ab x400,
e: Mucicarmine x400









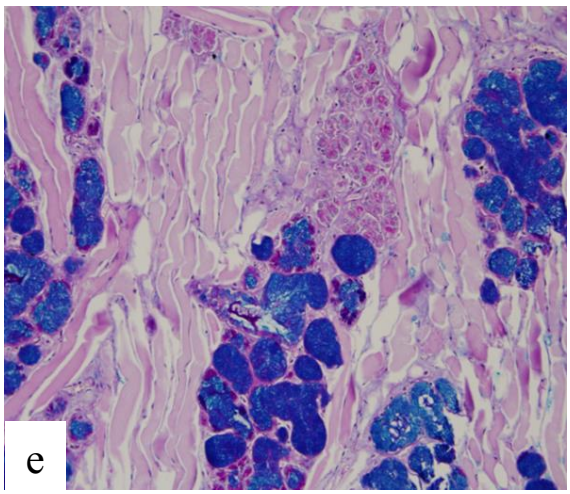


Figure 3　Gross and histological findings of the posterior lingual gland

Macroscopically, the size of the opossum tongue is 30 x 8 x 10 mm (a).

Microscopically, the posterior lingual glands are composed of two sets of minor salivary glands (b). Weber's glands (arrows) are consisted of seromucous acinar cells, and are mucous-rich mixed glands (c). von Ebner's glands (arrowheads) are located around of the circumvallate papillae, and are serous glands (d). In Weber's glands, mucous acinar cells are stained with alcian blue and/or PAS, and serous acinar cells is partly stained with PAS (e).

a: gross, b: HE x40, c: HE x400, d: HE x100, e: PAS-Ab x400

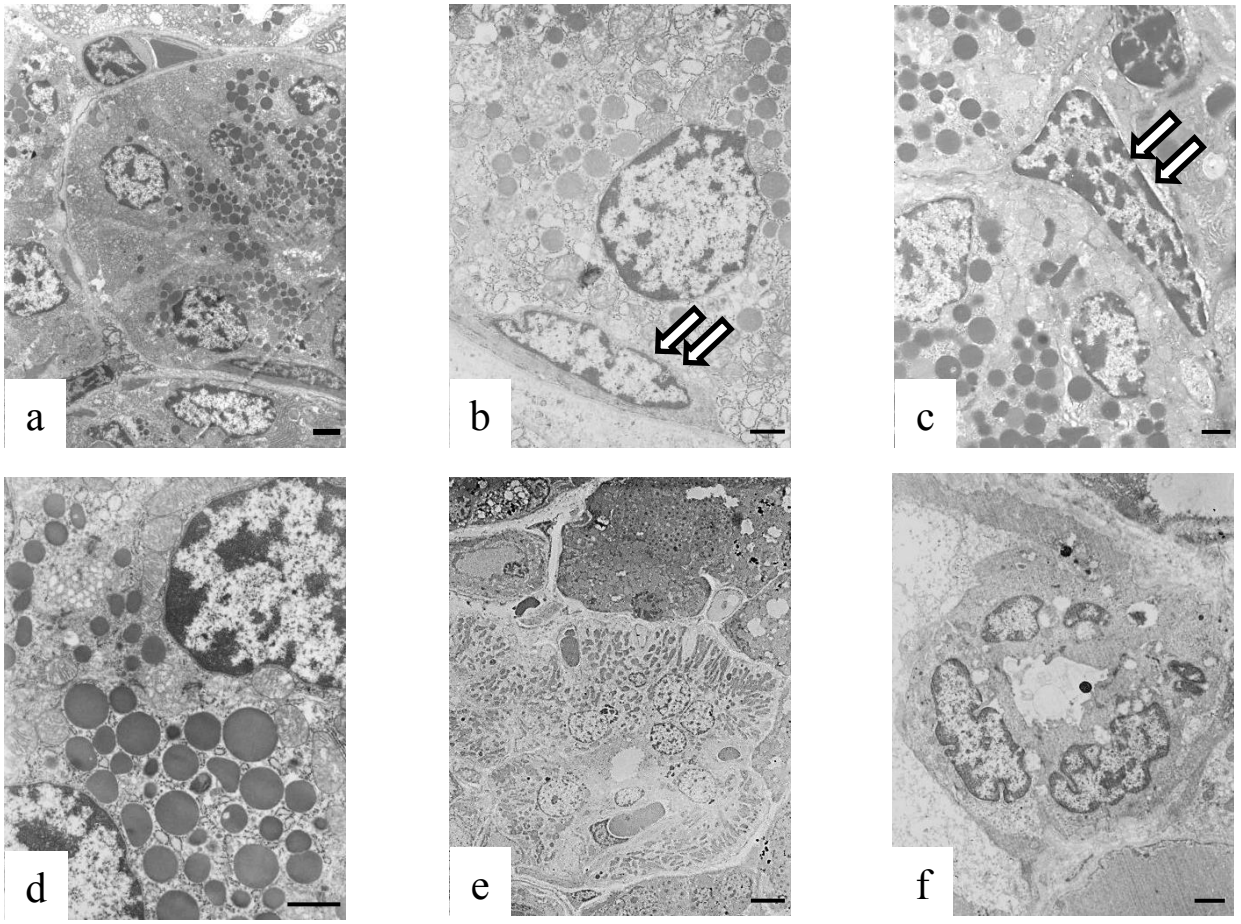


Figure 4　Ultrastructure of the parotid gland

Ultrastructually, the parotid gland acinar cells are pyramidal, and their nuclei exist at basal side (a). They have numerous mitochondria and rough ER throughout the cells, and myoepithelial cells (arrows) are along the outside of acinar cells (b, c). The secretory granules show homogeneous high electron density (d). The striated ducts are composed of columnar cells (e). The intercalated ducts are lined by cuboidal cells (f).

a: Secretory unit (bar= 5μm), b: ER (bar= 1μm), c: Myoepithelial cell (bar= 1μm),
d: Secretory granules (bar= 1μm), e: Striated duct (bar= 5μm), f: Intercalated duct (bar= 1μm)

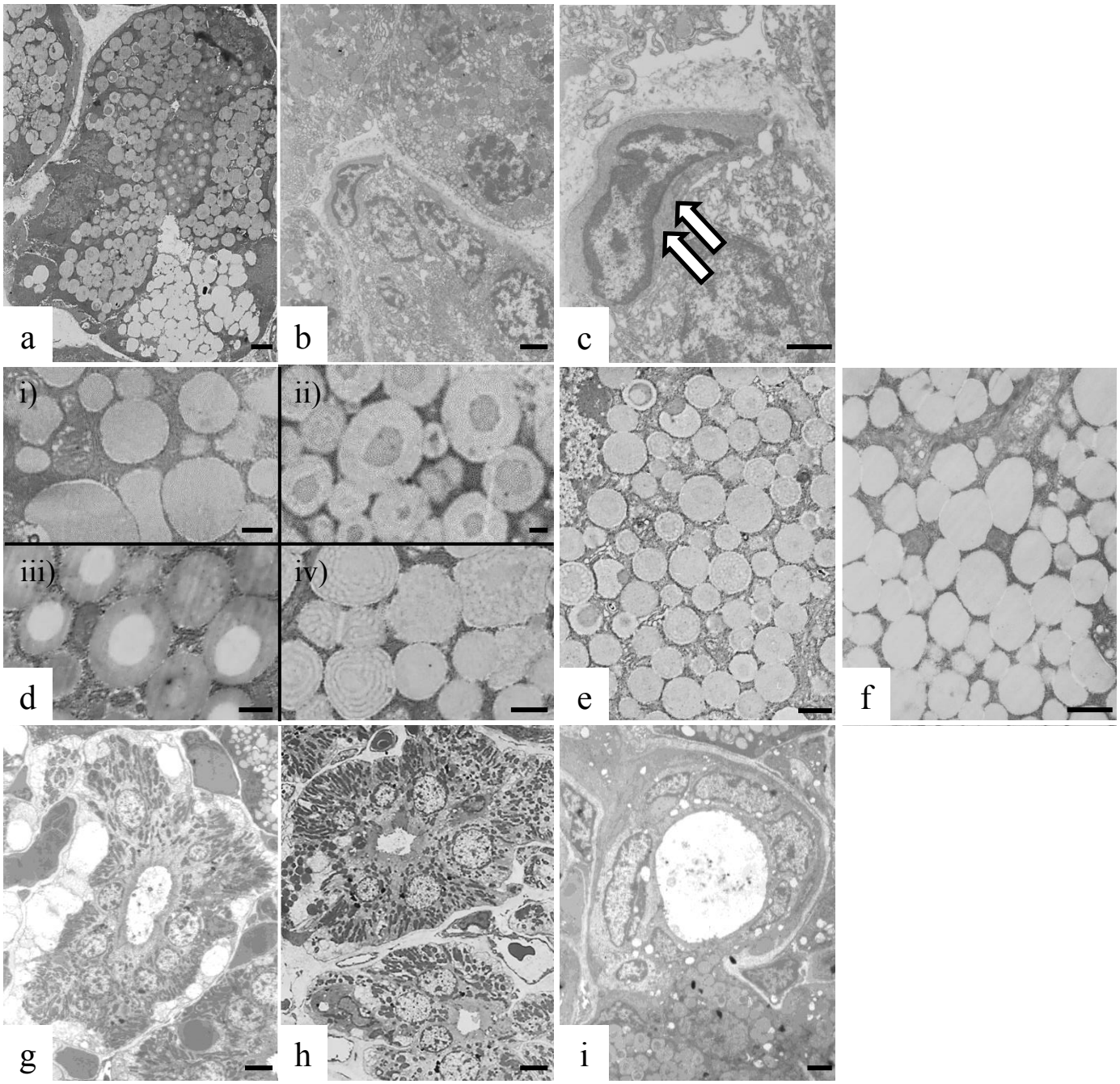


Figure 5 Ultrastructure of the submandibular gland

The secretory units are mixed glands, and possess the special serous acinar cells (a). The acinar cells have number of mitochondria and tubular or vesicular ER throughout the cells (b). The myoepithelial cell (arrows) contain abundant myofilaments (c). The secretory granules of serous are divided into i) homogeneous density type, ii, iii) two biphasic types and iv) annual ring types and these secretory granules of mucous cells show homogeneous low electron density (d-f). The striated ducts of submandibular glands are composed of columnar epithelial cells with or without basal infoldings, although those of the parotid glands were consisted of typical columnar epithelial cells with the infoldings. (g, h). The intercalated ducts are lined by cuboidal cells (i).

a: Secretory units (bar= 2μm), b: ER (bar= 2μm), c: Myoepithelial cell (bar= 1μm),
d: Serous secretory granules (bar= 500nm), e: Serous secretory granules (mixed type) (bar= 500nm),
f: Mucous secretory granules (bar= 1μm), g: Striated duct (bar= 2μm), h: Striated duct (bar= 2μm),
i: Intercalated duct (bar=2μm)