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Structural characteristics of goat (*Capra hircus*) parotid salivary glands

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Abstract

The structural characteristics of the parotid glands in small ruminants (goat, sheep) were observed and compared to those of a major laboratory animal, the mouse. Their parotid glands consist of the purely serous type. Ultrastructurally, the serous acini of goats and sheep were characterized by the presence of well-developed basolateral expansions of folds, which are characteristics of electrolyte- and water-transporting epithelium. Moreover in ruminants, unlike the mouse, the presence of numerous intercellular canaliculi as well as microvilli projecting into both the intercellular canaliculi and the lumina of the serous acini provided a large surface area for osmotic equilibrium and isotonic saliva secretion. Most of the secretory granules in goats and sheep contained peripherally located inclusions that showed dense reaction products for acid phosphatase. This indicates that most of the secretory granules undergo lysosomal degradation rather than secretion. An apocrine mode of secretion of some secretory granules was occasionally observed in some acini of goats and sheep but only exocytotic features were observed in mice. In the goat, the serous acini showed three morphologically different types, which might be an indication of different activity phases. Furthermore, alpha-smooth muscle actin-, and vimentin-positive myoepithelial cells were observed only around the serous acini and the intercalated ducts. From these findings, we consider that the structural characteristics of ruminant parotid glands might reflect their physiological role in the copious isotonic saliva secretion with a low protein concentration.

Keywords: acid phosphatase, goats, parotid gland, ultrastructure.

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Introduction

Ruminants are classified into three overlapping morphophysiological feeding types: concentrate selectors (CS: 40% of all ruminant species e.g., roe deer), grass and roughage eaters (GR: 25%, e.g. cattle and sheep) and intermediate feeders (IM: 35%, e.g. goats). The bigger parotid glands of CS and IM supply more saliva¹⁰. The total secretion of saliva per day has been estimated to be 6 to 16 liters in sheep and goats, and 100–190 liters in cattle². The normal composition of ruminant saliva is quite different from the saliva of monogastric animals. It is an isotonic, bicarbonate phosphate buffer secreted in large quantities with high pH. This well-buffered solution is necessary for neutralizing acids formed by fermentation in the rumen to maintain the acid-base equilibrium of the ruminal contents^{5,14}. There are marked differences in the protein contents in saliva between nonruminants and ruminants, as there are very low values for parotid saliva protein concentrations (0.1 ± 0.1 mg/mL) in both sheep and goats, whereas these values are higher in humans (1.0 to 2.0 mg/mL) and similar values have been noted for rodents^{6,7,11}.

Mammalian salivary glands consist of secretory endpieces and duct systems that vary in structure among different types of salivary glands and among different species. The secretory endpieces of the parotid salivary glands (PGs) are of the pure serous type in the goat and sheep but of the seromucous type in the dog^{17,21}. Furthermore, the distribution of myoepithelial cells (Mecs) in the salivary glands exhibits striking diversity among species. They are reported to be found around the acini and intercalated ducts (Icds) in goats, dogs and horses^{16,17}. However, in rabbits, it is reported that myoepithelial cells are not detected around the acini, but are found around the Icds¹⁵.

The structure of salivary glands in mammals has been thoroughly studied^{8,12}. However, only a few reports on farm animals such as goats have

been published. Thus, the present study was undertaken to describe the histological, histochemical and ultrastructural characteristics of adult goat PGs, including the acini and the elaborate duct system. Moreover, we tried to determine the ecological correlation between the structure of goat PG cells and their physiological role in the copious secretion of saliva with a low protein concentration.

Materials and Methods

Animals: The present study was carried out in accordance with the Hokkaido University guideline for the care and use of animals. PGs were obtained from 6 male goats (*Capra hircus*) aged from 6 to 24 months. The PGs of 2 male sheep and 4 male mice (MRL/MpJ) were used as controls for demonstrating the ultrastructural differences between ruminant and nonruminant species. Animals were euthanized by exsanguination through the carotid arteries under anesthesia, using xylazine (0.3 mg/kg, i.m.) and pentobarbital sodium (6 mg/kg, i.v.) for goats and sheep and pentobarbital sodium (40 mg/kg, i.p.) for mice.

Tissue preparation: For light microscopic examination, freshly collected specimens from the central parts of the PGs were fixed immediately in either 10% buffered neutral formalin for 24 hr, or 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB), pH 7.4, and stored for at least 2–4 hr. They were then embedded in paraffin using the usual histological techniques. Histological sections 3 μ m thick were stained with hematoxylin-eosin (HE) for cellular morphology analysis, the periodic acid Schiff's (PAS) technique and alcian blue (AB) stain at pH 1.0 for detection of neutral and acidic mucopolysaccharides, respectively. To examine the distribution of Mecs¹, immunohistochemical studies were carried out on the paraffin sections by the streptavidin-biotin technique, using the

following antibodies: 1) polyclonal rabbit antisera to α -SMA (RB-9010, Thermo Scientific, Fremont, CA, USA), and 2) polyclonal goat antisera to vimentin (V4630, Sigma Chemical Co., Saint Louis, Missouri, USA). Antigen retrieval was conducted for detection of Mecs (Table 1). Endogenous peroxidase was blocked by incubating sections in 3% H_2O_2 in absolute methanol for 30 min at 4°C, followed by flushing with water and incubation with 10% normal serum (goat serum for α -SMA staining and donkey serum for vimentin staining) for 1 hr at room temperature. The sections were incubated overnight with the specific primary antibody diluted in 1.5% BSA/0.1 M phosphate-buffered saline (PBS) (pH 7.2) (Table 1). For negative control sections, PBS was used instead of the primary antibody. Sections were washed in PBS, followed by incubation with biotin-conjugated goat anti-rabbit IgG antiserum (Histofine kit, Nichirei Corporation) for 60 min. Sections were then washed three times in PBS, followed by incubation with streptavidin-peroxidase conjugate (Histofine kit, Nichirei Corporation) for 30 min. The streptavidin-biotin complex was visualized with 3,3'-diaminobenzidine tetrahydrochloride (DAB)- H_2O_2 solution for 3 min. Sections were next washed in distilled water, and finally counterstained with hematoxylin.

For transmission electron microscopical (TEM) examination, small pieces of PGs from goats, sheep and mice were fixed with 3% glutaraldehyde (GTA) in 0.1 M PB (pH 7.4) for 2 hr. The tissues were post-fixed for 1 hr with 1% osmium tetroxide (OsO_4) in 0.1 M PB. Then the samples were dehydrated in ascending grades of ethyl alcohol, and embedded in epoxy resin (Quetol 812 Mixture) (Nishin EM, Co.,

Ltd., Tokyo, Japan). Ultrathin sections (70 nm) were double stained with uranyl acetate and lead citrate. All samples were analyzed and photographically captured using a JOEL transmission electron microscope operated at 80 kV (JEM 1210, JEOL, Tokyo, Japan).

For histochemical examination, acid phosphatase (AcPase) in goat PGs was assayed by histochemical testing. For the demonstration of AcPase activity, 40–100 μ m-thick sections were fixed in a mixture of 1.25% GTA and 2% paraformaldehyde (PFA) in 0.1 M sodium cacodylate buffer (CB) with 8% saccharose at 4°C for 45 min. Following fixation, the specimens were rinsed in 0.1 M CB with 8% saccharose, and stored overnight at 4°C. Then the sections were incubated for 20 min at 37°C in a medium containing 50 mM acetate buffer at pH 5.5, 6.5 mM sodium glycerolphosphate, 3.5 mM lead nitrate and 0.2 M sucrose. Moreover, some specimens used as negative controls were incubated in the above medium to which 0.01 M sodium fluoride had been added²¹. Then all specimens were rinsed in 0.1 M CB with 8% saccharose at 4°C for 30 min followed by post-osmification, dehydration and Quetol-mixture embedding. Semithin sections were stained with toluidine blue, and ultrathin sections (70 nm) were double-stained with uranyl acetate and lead citrate. All samples were analyzed and photographically captured using a transmission electron microscope.

Morphometrical examination: To measure the sizes of the lumina of the secretory endpieces, light micrographs of five sections of each animal were prepared. Then both the areas of the secretory endpieces and those of lumina were

Table 1. The antibodies used, working dilution, and antigenic recovery.

Antibody	Dilution	Antigenic recovery	Incubation time and temperature
α -SMA	1 : 1,000	Citrate buffer (pH 6.0)	105°C, 20 min
Vimentin	1 : 700	0.05% Trypsin	37°C, 20 min

measured using Image J (free software from NIH), and the percentages of the luminal areas against the secretory endpieces areas were calculated. Furthermore, by using five transmission electron microscopic TEM pictures, the numbers of secretory granules (SGs)/serous cell were counted. Moreover, the density of the nucleus was analyzed according to its chromatin contents.

In TEM, the degrees of development of plasma membrane specialization and cellular components (such as basal infoldings [BI], intercellular canaliculi [IcC], microvilli [MV], Golgi complexes, and mitochondria) were calculated using four grades: non-developed (score 0), normal (score +1), developed (score +2), and well developed (score +3). We observed four TEM pictures in each category and the means of the scores were calculated. The results of semi-quantitative analysis were expressed as follows; score 0 (–), score 0.1–0.5 (±), score 0.6–1.0 (+), score 1.1–2.0 (++) , score 2.1–3.0 (+++).

Statistical analysis: The ratios of the luminal areas/secretory endpieces areas and the numbers of the SGs were expressed as mean \pm SEM. We used both Kruskal-Wallis test to compare the ratios of the luminal areas/secretory endpieces areas among different species also to compare such ratios as well as the numbers of the SGs among different secretory phases of goat PG, and Scheffé's method for multiple comparisons when a significant difference was observed ($P < 0.05$).

Results

Comparison of the histological features of the PGs between small ruminants (goat, sheep), and rodents (mouse)

Light microscopy

The PGs in the goat, sheep and mouse were

found to be of the purely serous type. They consisted of a compound alveolar gland in goats and mice (Fig. 1 A, C) but of a compound tubular type in sheep (Fig. 1 B). In all species, the cytoplasm of the secretory endpieces and the proximal portions of the Icd showed the presence of granules. On the other hand, the other ductal epithelial cells were devoid of these granules. The granules showed moderate PAS-positive reactions in goats and sheep (Fig. 1 D, E) and intense reactions in mice (Fig. 1 F) but the reaction for AB stain was negative in all species (Fig. 1 G–H). Moreover, the serous granules were distributed throughout the apical cytoplasm of the serous cells in goats and sheep (Fig. 1 D, E) but were distributed throughout the entire cytoplasm in mice (Fig. 1 F). The lumina of the secretory endpieces differed among the studied species (Fig. 1 D–F). The secretory endpieces had small lumina ($2.3 \times 10^2 \pm 1.9 \times 10^2 \mu\text{m}^2$) in the mouse, moderate lumina ($1.7 \times 10^3 \pm 3.2 \times 10^3 \mu\text{m}^2$) in the goat and large lumina in sheep ($9.6 \times 10^3 \pm 4.7 \times 10^3 \mu\text{m}^2$) (Table 2). The ratio of the lumen area/endpiece area in sheep was significantly higher than those of the other species (Table 2). Moreover, in the PGs of both the goat and sheep a few mucous secreting cells with intense positive reactions for both PAS and AB stains appeared in the distal portions of the interlobular ducts, but such positively reacting cells were not found in the mouse (Fig. 1 G–I). The number of these cells was obviously found to be increased as the ducts directed toward the parotid papilla in sheep and goats (data not shown).

Electron microscopy

The secretory endpieces in all studied species were composed of pure serous secretory cells (Fig. 2). In goats and mouse they were of the compound alveolar type (Fig. 2 A, C); however, those of sheep were of the compound tubular type (Fig. 2 B). The PGs of both goats and sheep had more intercellular canaliculi (IcC) between the adjacent acinar cells than those of

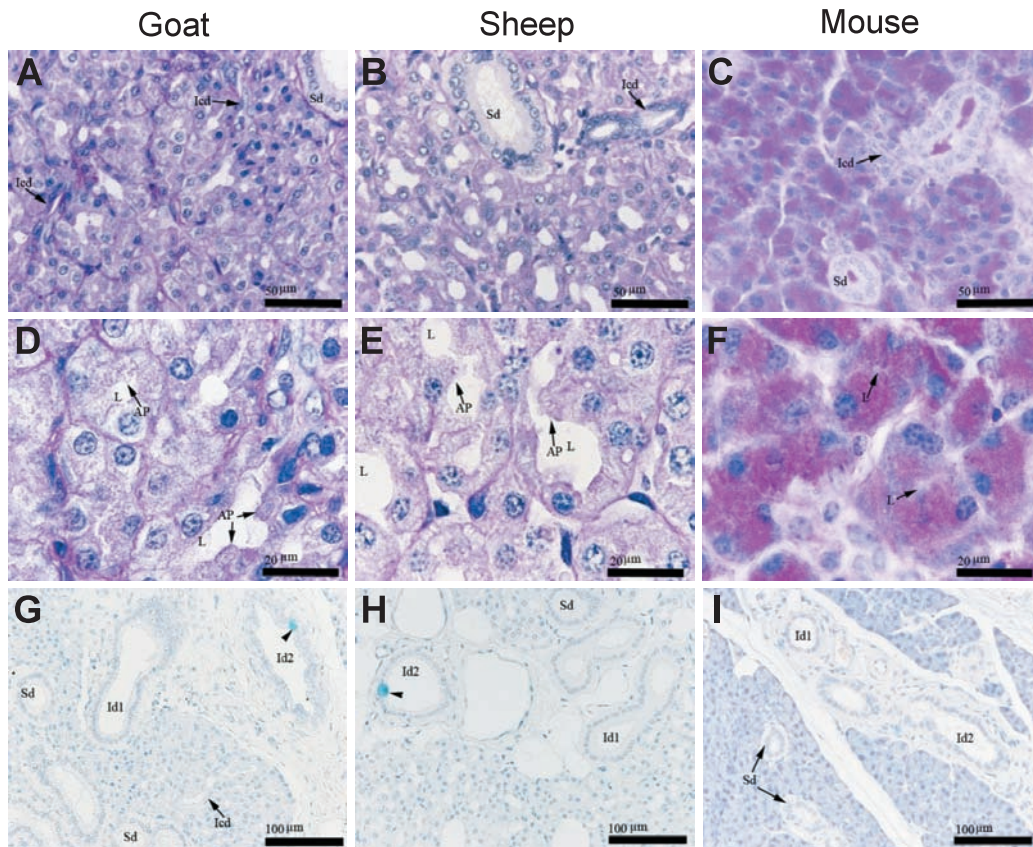


Fig. 1. Light microscopic structure of the goat (panel A, D, G), sheep (panel B, E, H) and mouse (panel C, F, I) parotid gland. PAS positive granules in secretory endpieces of moderate reaction in the PG of goat and sheep (panel A, B, D, E) and of intense reaction in that of mouse (panel C, F, I), and apocrine like protrusions (AP) into the lumen (L) are observed (panel E). Note that in both goat and sheep a few mucous secreting cells (arrow heads) appear in the distal portion of interlobular duct (Id2) but were absent from that of intercalated duct (Icd), striated duct (Sd) and the proximal portion of interlobular duct (Id1) (panel G, H). All of the mouse parotid gland ducts lack these cells (I). PAS reaction (A-F), alcian blue stain (panel G-I).

Table 2. Comparison of the ratios of the lumen area/endpiece area among the PG of different species (goat, sheep and mice)

Animal	Lumen area (μm^2)	Endpiece area (μm^2)	Ratio of lumen area/endpiece area (%)
Goat	$1.7 \times 10^3 \pm 3.2 \times 10^3$	$3.2 \times 10^4 \pm 1.2 \times 10^4$	6.2 ^{b, *}
Sheep	$9.6 \times 10^3 \pm 4.7 \times 10^3$	$4.2 \times 10^4 \pm 1.6 \times 10^4$	22.3 ^{a, c, *}
Mice	$2.3 \times 10^2 \pm 1.9 \times 10^2$	$3.2 \times 10^4 \pm 8.3 \times 10^4$	0.4 ^{b, *}

* Significant species difference (Kruskal-Wallis test, $P < 0.05$)

^{a, b, c} Significant difference with goat, sheep, and mice, respectively (Scheffé's method, $P < 0.05$)

the mouse (Fig. 2 A-C). They occupied approximately one-third of the epithelial volume in both goats and sheep. Moreover, the serous cells in both goats and sheep showed well-developed lateral intercellular infoldings or interdigitations of the plasma membrane (Fig. 2

D, F). Additionally, in both sheep and goats, numerous long microvilli were seen projecting into the canaliculi as well as the lumina of the acini (Fig. 2 D, E). On the other hand, they were shorter and fewer in mice (Fig. 2 F). In goats, the intercellular spaces and canaliculi were

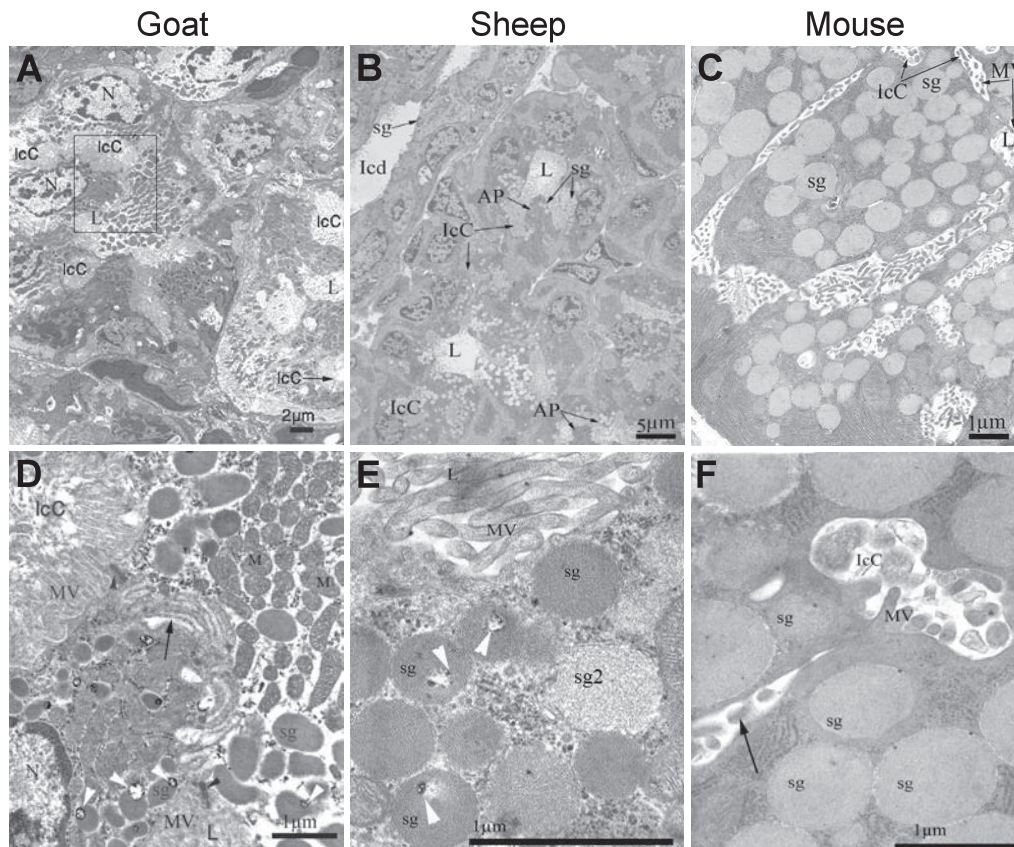


Fig. 2. Transmission electron micrographs of the secretory endpieces of the parotid gland of the goat (panel A, D “higher magnification to the rectangle in panel A”), sheep (panel B, E) and mouse (panel C, F), showing compound alveolar gland (panel A, C) and compound tubular gland (panel B). Note the serous cell nucleus (N), lumen (L), intercellular canaliculi (IcC), microvilli (MV), lateral intercellular infoldings and space (arrow in panel D, F respectively), intercalated duct (Icd), secretory granules (sg), less electron dense granules (sg2), peripherally located inclusions (white arrowheads), mitochondria (M), junctional complex (black arrowheads), and apocrine-like protrusions (AP).

sealed by well-developed junctional complexes (Fig. 2 D). The apical cytoplasm of the serous cells in both sheep and goats showed the presence of numerous membrane-bound SGs with homogenous matrices and assorted sizes (Fig. 2 D, E). However, in mice such granules were distributed all over the cytoplasm (Fig. 2 C, F).

In the goat and mouse only one type of SG could be observed. The matrices of the SGs in the goat were quite electron dense (Fig. 2 D); however, that in the mouse were less electron dense (Fig. 2 F). In sheep, two types of secretory granules with variable electron density were identified. The matrix of the first type was quite

electron dense and they were relatively small but that of the second type was less electron dense and they were larger (Fig. 2 E). Moreover, in goats and sheep, but not mice many of the SGs with the quite electron dense-matrix showed the presence of peripherally located spheroids or inclusions with low electron density, and some of the inclusions showed the presence of dark spots (Fig. 2 D-F).

Lysosomes in the goat PG

Fig. 3 shows the results for acid phosphatase enzyme activity in the goat PG. Ultrastructurally, acid phosphatase was found in lysosome-related structures (GERL; Golgi-associated endoplasmic reticulum lysosome). It

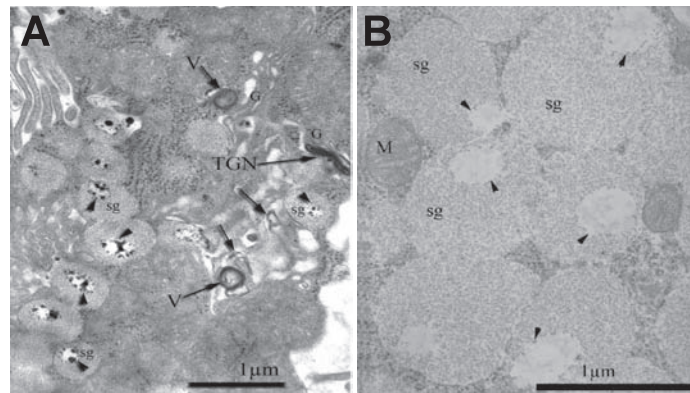


Fig. 3. Transmission electron micrographs of the goat parotid gland. A, B. Serous acini incubated in acid phosphatase medium using β -glycerophosphatase as the substrate (panel A) with addition of 0.01 M sodium fluoride to the incubating media (panel B). Note mitochondria (M), secretory granules (sg) with peripherally located inclusions (arrow heads) that showed dense reaction products for acid phosphatase in panel A, and the reaction is completely inhibited on panel B. Acid phosphatase reaction products are also observed in panel A in some lysosome-like vesicles (V) near the Golgi complexes (G) as well as in some inner Golgi cisternae (arrows) and the trans-Golgi network (TGN).

was located in various spheroidal bodies as dense reaction products that could be described as primary and secondary lysosomes as well as in some inner Golgi cisternae, and in some lysosome-like vesicles near the apparatus. Moreover, most of the trans-Golgi network (TGN) displayed intense acid phosphatase reactivity. Materials resembling the dense material were often observed in the lumen as well as the intercellular spaces (Fig. 3 A). The AcPase reaction was completely inhibited by using 0.01 M sodium fluoride with the incubation media, a method that was previously used for negative control²¹). The SGs with peripheral-less-electron dense inclusions suggested the lack of dense reaction products (Fig. 3 B).

Different types of serous acini (SA) in goat PGs

In light microscopic observation of the goat parotid glands, the SA within the same gland exhibited three different morphological features. We classified the SA into three phases named the first, second, and third phases. In the first phase, the cells showed highly condensed apical granules with basally situated spherical vesicular nuclei, and the acini had relatively small lumina ($3.3 \times 10^2 \pm 2.2 \times 10^2 \mu\text{m}^2$). In the second phase, less vesicular nuclei with

moderately sized lumina ($2.0 \times 10^3 \pm 1.1 \times 10^2 \mu\text{m}^2$) were observed. In the third phase, the acini showed disrupted ill-defined large lumina ($7.4 \times 10^3 \pm 4.7 \times 10^2 \mu\text{m}^2$) with dark nuclei that sometimes bulged into the lumina (Fig. 4 A, B). The ratios of the lumen area/endpiece area among different maturation activity phases showed significantly different values (Table 3).

As with the light microscopical observations, ultrastructurally the SA of the goat PG displayed marked variation in size and showed three phases. In the first phase, the acinar cells enclosed a narrow, regular round lumen with numerous apical long microvilli projecting into it (Fig. 4 C). The apical cytoplasm showed the presence of highly condensed SGs with short segments of rER and free ribosomes sparsely distributed in it as well as numerous mitochondria, but Golgi complexes were rarely observed. Well-developed plasma membrane specializations were observed, including the presence of basal infoldings as well as relatively numerous IcC between the adjacent acinar cells with extensive microvilli. The nucleus was basally located and was electron lucent (Fig. 2 A, D). In the second phase, the acinar cells enclosed an irregular lumen of moderate size with apical

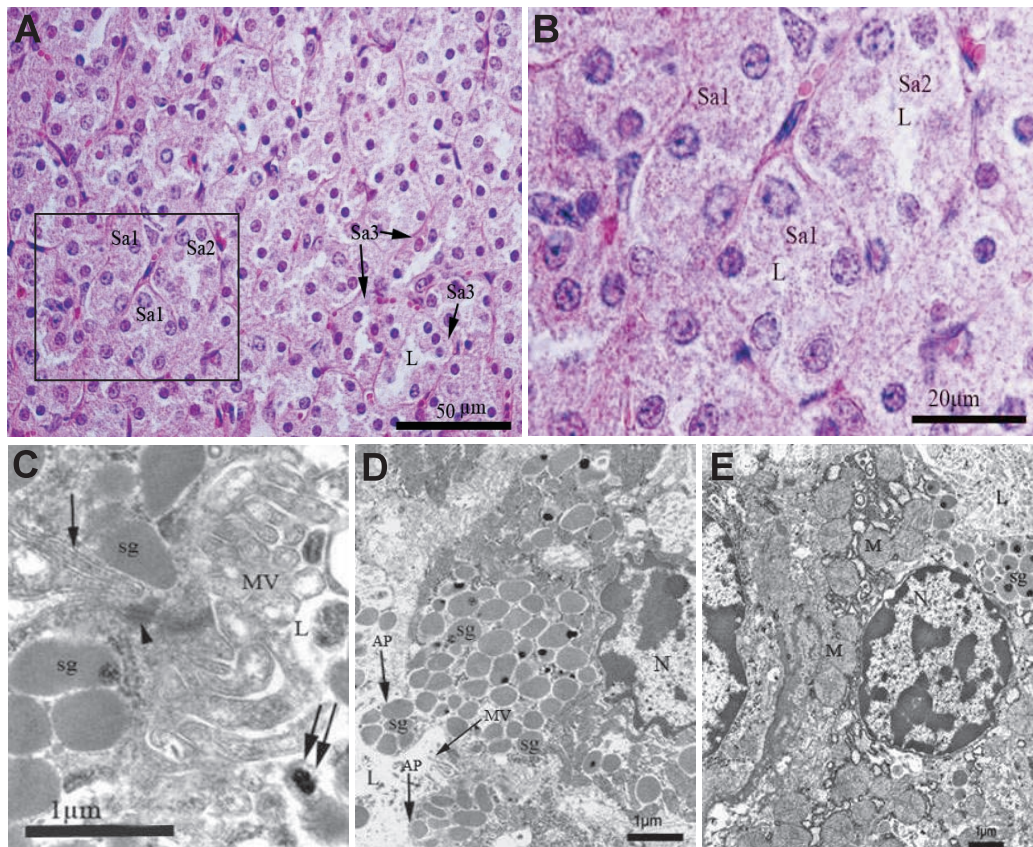


Fig. 4. Light (panel A, B), and transmission electron (panel C-E) micrographs of the secretory phases in the goat parotid gland. Panel A, B): Showing the parotid acini with different phases of activity, first phase (Sa1); second phase (Sa2), and third phase (Sa3), lumen (L). Hematoxylin-Eosin stain. In panel C, D, and E): Showing the apical cytoplasm of serous cells in different activity phases (panel C: First phase, panel D: Second phase, and panel E: Third phase). Note the well developed lateral intercellular interdigitations (arrows), junctional complex (arrowheads), dense materials within the lumen (double arrows); apocrine like protrusions (AP); the presence of microvilli (MV) bulged into the lumen (L). The MV are numerous, long in the first phase (panel C), and few, short in the second phase (panel D), also the apical cytoplasm in the first and second phases (panel C, D) showed numerous secretory granules (sg) but the serous cells in third phase (panel E) showed the lack of both MV and secretory granules, and the later could only be observed in the lumen. The serous cell nucleus (N) is centrally located in D, apically located, near the lumen in third phase (panel E).

Table 3. Comparison of both the ratios of the area of the lumen/acini, and the numbers of secretory granules/serous cell among the maturation activity phases in goat PG.

Secretory phases	Lumen area (μm^2)	Endpiece area (μm^2)	Ratio of lumen area/endpiece area (%)	Number of SG/serous cell
First phase	$3.3 \times 10^2 \pm 2.2 \times 10^2$	$2.7 \times 10^4 \pm 1.0 \times 10^4$	0.6 ^{b, c, *}	$56.8 \pm 5.8^{\text{b, c, *}}$
Second phase	$2.0 \times 10^3 \pm 1.1 \times 10^2$	$3.2 \times 10^4 \pm 1.2 \times 10^4$	1.6 ^{a, c, *}	$42.0 \pm 5.1^{\text{a, c, *}}$
Third phase	$7.4 \times 10^3 \pm 4.7 \times 10^2$	$4.1 \times 10^4 \pm 1.3 \times 10^4$	6.8 ^{a, b, *}	$1.3 \pm 1.8^{\text{a, b, *}}$

* Significant phases difference (Kruskal-Wallis test, $P < 0.05$)

^{a, b, c} Significant difference with first, second and third phases, respectively (Scheffé's method, $P < 0.05$)

microvilli projecting into it. These microvilli were less numerous and shorter than those of the storage phase. Numerous apocrine-like

protrusions of the apical cytoplasm containing condensed SGs were frequently observed bulging into the lumen of the acinus. The plasma

membranes of these protrusions were smooth and without microvilli (Fig. 4 D). Golgi complexes were rarely observed. Organelles like the rER and free ribosomes as well as mitochondria were less numerous than in the first phase and distributed mainly in the basal cytoplasmic portion of the acinar cells. The basal part of the acini showed more-developed basal infoldings but less numerous IcC with less-developed and shorter microvilli than in the first phase (Fig. 4 D). The nucleus was centrally located and less electron lucent than that of the first phase. In the third phase, the acinar cells enclosed a large, irregular, ill-defined lumen with very few and shorter microvilli than in the second phase and sometimes it was absent (Fig. 4 E). The SGs were found to be either very few in the apical cytoplasm or absent and sometimes observed only in the lumen. Well-developed Golgi complexes and numerous mitochondria were frequently observed around the nucleus. The nucleus was apically located and sometimes bulged into the lumen and had a more electron-dense appearance than those of the previously mentioned phases (Fig. 4 E).

Neither basal infoldings nor IcC were well developed. The numbers of secretory granules showed significantly different values among the three phases (Table 3).

From both the light and transmission electron microscopic observations, we concluded that the first phase could be considered a storage phase, the second phase a secretory phase, and the third one an exhaustion phase.

The light and transmission electron microscopic observations of the secretory phase in goat PG acini are summarized in Table 4.

Several characteristic structures in the goat PG

The transition from acinar cells to those of Icds was abrupt. A few small SGs were observed in the acinar sides of the intercalated ducts in all species (data not shown). In the goat, some of the Icd cells also had apocrine-like protrusions of their SGs into the lumen (Fig. 5-A). The characteristic pleomorphic apocrine-like protrusions of the apical membranes of the serous and intercalated duct cells that were observed in the goat PG were also observed in

Table 4. Phases of activity of the secretory acini in goat parotid gland.

Methods	Criteria	First phase	Second phase	Third phase
LM	Lumen	Regular, small	Irregular, moderate	Ill defined, large
	Nucleus	Vesicular, basally located	Less vesicular, centrally located	Dark, apically located
TEM	Granules	+++	++ (with apocrine like protrusions)	±
	BI	++	+++	—
	IcC	+++	++	—
	Apical microvilli	+++	+	±
	Golgi complexes	+	+	+++
	Mitochondria	+	++	+++
	Nucleus	Electron lucent	Less electron lucent	More electron dense

LM: Light microscopy; TEM: Transmission electron microscopy, BI: Basal infoldings, IcC: Intercellular canaliculi. The degrees of development of plasma membrane specialization and cellular components were calculated using four grades: non-developed (score 0), normal (score +1), developed (score +2), and well developed (score +3). Finally, the means of scores of semi-quantitative analysis were applied to these gradings; score 0 (—), score 0.1-0.5 (±), score 0.6-1.0 (+), score 1.1-2.0 (++), score 2.1-3.0 (+++).

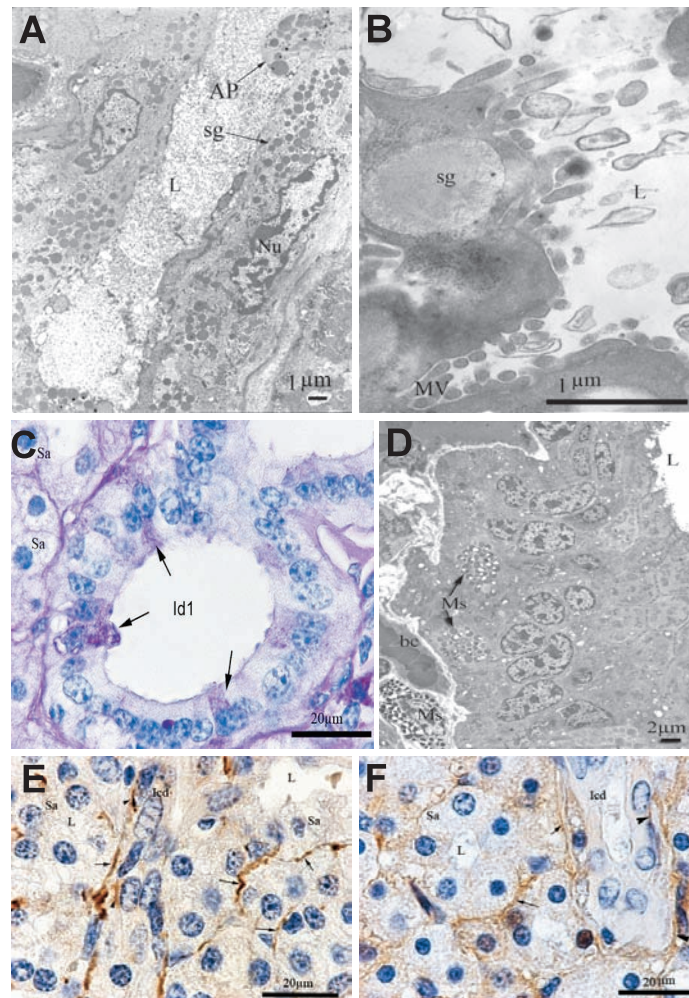


Fig. 5. Transmission electron micrographs (panel A, B, D), and Light micrographs of the goat (panel A, C, D, E, F), and mouse (panel B) parotid gland. Panel A): Showing the intercalated duct wall with flattened nucleus (Nu), apically located secretory granules (sg), and apocrine like protrusions (AP) into the Lumen. Panel B): Showing the apical cytoplasm of the serous acini of mouse parotid gland. Note very few, short microvilli (MV), discharge of the secretory granules (sg) into the lumen (L) by exocytosis. Panel C): Showing PAS reaction in goat parotid gland. Note the serous acini (Sa), proximal portion of the interlobular duct (Id1) with numerous PAS-positive migratory granulated cells (arrows). Panel D): Showing the interlobular duct wall of goat parotid gland. Note the lumen (L), blood capillary (bc), and mast cells (Ms) migrating through the duct wall and in the interlobular connective tissue. Panel E, F): Immunohistochemical staining of the goat parotid gland with α -SMA (Panel E), and vimentin (Panel F) antisera. Note the crescent-shaped positive immunostaining of myoepithelial cell processes (arrows), (arrowheads) around the serous acini (Sa), and intercalated duct (Icd), respectively.

the sheep PG (see, Fig. 2 B). On the other hand, the discharge of the SGs into the lumen in the mouse was found to occur via exocytosis (Fig. 5 B) with no evidence of apocrine mode of secretion.

Interestingly, in the goat PGs, a few migratory granulated cells with strongly PAS-positive granules were found among the

lining epithelial cells of both the striated ducts and the proximal portions (acinar side) of the interlobular ducts (Fig. 5 C). Such cells were ultrastructurally identified as mast cells with numerous electron-dense granules. These intraepithelial mast cells were also found in the stroma surrounding both ducts (Fig. 5 D). However, such cells could not be detected in the PGs of either the sheep or mouse. Moreover, in

all species, some lymphocytes were observed migrating through the walls of both ducts. Plasma cells were also observed in the stroma around the acini and ductal system (data not shown). In goat PGs, Mecs with positive immunostaining for both α -SMA (Fig. 5 E) and vimentin (Fig. 5 F) antisera were only observed around the serous acini and the proximal portions (acinar side) of Icds. The light and transmission microscopic observations of the goat, sheep and mouse PGs acini and the elaborate duct system are summarized in Table 5.

Discussion

The salivary glands of mammals are known to exhibit striking diversity in histology, ultrastructure and histochemistry¹³⁾. To our

knowledge, few studies have comparatively analyzed PGs among ruminant species. Therefore, structural correlations with physiological functions could not be discerned. Goats are intermediate-feeder-type ruminants¹⁰⁾, so they may be an ideal model to study such correlations, especially as there may be some correlations between the structure and nature of the diet. In the present study we found that the structure of the goat PG had some features of both CS- and GR-feeding ruminants since it was a compound alveolar gland, as in the majority of CS ruminants¹⁹⁾. However, a compound tubular gland has been reported for some GR ruminants like the sheep²¹⁾, similar to that described for the sheep PG in the present study. Furthermore, the present study revealed significant differences in the ratios of the lumen area/secretory endpiece area between the sheep and goat. The secretory endpieces contain large lumina in sheep ($9.6 \times$

Table 5. Light and transmission electron microscopical features of the parotid gland in goat, sheep, and mice

Criteria		Goat	Sheep	Mouse
Parotid gland type		Compound alveolar	Compound tubular	Compound alveolar
Secretory end pieces	<u>Lumen size</u>	++	+++	+
	<u>IcC</u>	+++	+	+
	<u>MV</u>	+++	+++	+
PAS positive SGs in the cytoplasm of serous cells	<u>Location</u>	Apically located	Apically located	Located throughout the cytoplasm
	<u>Number and intensity</u>	++	++	+++
SGs with peripheral inclusions		+	+	—
Apocrine like protrusion		+	+	—
Mucous secreting cells in the duct	<u>Icd, Sd, Id1</u>	—	—	—
	<u>Id2</u>	+	+	—
	<u>Eds</u>	+++	+++	—
Mast cells	<u>Sd, Id1</u>	+	Not detected	Not detected

IcC: Intercellular canaliculi, MV: Microvilli, SGs: Secretory granules, Icd: Intercalated duct, Sd: Straited duct, Id1: the proximal portion of interlobular duct, Id2: the distal portion of interlobular duct before its opening in the excretory ducts (Ed). PAS: Periodic acid Schiff's reaction.

The degrees of development of plasma membrane specialization and cellular components were calculated using four grades: non-developed (score 0), normal (score +1), developed (score +2), and well developed (score +3). Finally, the means of scores of semi-quantitative analysis were applied to these gradings; score 0 (—), score 0.1-0.5 (\pm), score 0.6-1.0 (+), score 1.1-2.0 (++) , score 2.1-3.0 (+++).

$10^3 \pm 4.7 \times 10^3 \mu\text{m}^2$) but moderately sized lumina ($1.7 \times 10^3 \pm 3.2 \times 10^3 \mu\text{m}^2$) in goats. The serous cells in the goat showed also the presence of only one type of SG with a quite electron dense matrix; however, in sheep some SGs with a less electron dense matrix were also observed in addition to those observed in goats. Furthermore, some of the SGs with the quite electron dense matrix showed the presence of peripherally located low-electron-density inclusions in both sheep and goats, similar to what was reported for GR-feeding ruminants^{19,21}. Moreover, the secretory endpieces of both the goat and sheep PG showed well-developed intercellular interdigitations as well as secretory canaliculi, which are needed for the secretion of large amounts of fluids, similar to what was reported for GR-feeding ruminants^{18,21}. On the other hand, in CS-feeding ruminants, the organelles of the parotid acinar cells are more typically composed of protein-secreting cells, and the secretory canaliculi and intercellular spaces are not predominant^{9,19}.

Saliva is the first biological medium to confront external materials that are taken into the body as food or drink or inhaled volatile substances³. In goats, many mast cells as well as lymphocytes were observed migrating through the walls of both striated and interlobular ducts. Such intraepithelial mast cells were also reported in the PGs of wild ruminants and sheep^{19,21}. Moreover, numerous plasma cells were also observed in the interlobular connective tissues as well as between the acinar cells. These results suggested the protective effect of saliva and the role of saliva as the first line of oral defense.

At the ultrastructural level, the SA of both goats and sheep showed well-developed basolateral expansions of folds, which were characteristic of electrolyte- and water-transporting epithelium, and copious secretion of saliva. Moreover, goat saliva is reported to be isotonic¹⁴, and a large surface area is required

for osmotic equilibrium. This was apparent in our observation of the presence of numerous IcC between the adjacent acinar cells, which occupied approximately one-third of the epithelial volume, with numerous long microvilli projecting into both the IcC and the lumen of the SA.

It has been suggested that parotid gland granule morphology is a fingerprint of the species, and different species seem to have different granule morphologies²⁰. Our observations support this suggestion to some extent, as some of the SGs of sheep and goats, but not mice, showed the presence of peripheral inclusions with a homogeneous matrix having low electron density. Such inclusions have been also reported in some ruminant species, including sheep^{19,21}, but were not observed in other ruminant species^{18,19}.

Furthermore, despite observing dense spots in some of the peripherally located inclusions in both sheep and goats under the same experimental conditions, we considered these spots to be artifacts resulting from the use of 0.1 M phosphate buffer during the fixation and post-fixation where the phosphate of the buffer may be precipitated by the lead ions. At the same time, these spots were not observed using 0.1 M CB and 0.01 M sodium fluoride as an inhibitor of AcPase activity.

Despite the fact that the acinar cells of goats and sheep had well-developed endoplasmic reticulum, large Golgi complexes and numerous SGs, which were considered to be unique characteristics of protein-secreting cells, our observations were in line with the previously reported lower values for parotid saliva protein concentrations in both sheep and goats^{9,11}. Considering our results, this could be due to the combination of a number of factors. These include the presence of SGs that were restricted to the apical cytoplasm of the serous cells in goats and sheep; however, those in the mouse were numerous and distributed throughout the

cytoplasm, and not all acinar cells in goats had SGs in their apical cytoplasm, where three secretory phases were detected in their SA. Moreover, not all the SGs were found to be secreted into the lumen, as most of them showed the presence of peripheral inclusions that had dense reaction products for AcPase and might be considered to be secondary lysosomes, thus suggesting that most SGs undergo lysosomal degradation rather than secretion. On the other hand, the SA of the mouse had more numerous SGs than those of the goat and sheep, which were secreted into the lumen with no signs of lysosomal degradation. These results also explained the higher values for parotid saliva protein concentrations reported for rodents^{6,7}.

The ultimate fate of many secondary lysosomes is uncertain, but the presence of material resembling the dense materials in these lysosomes in both the lumen and intercellular spaces suggests possible luminal extrusion of some of their indigestible contents.

A characteristic apocrine mode of secretion of some of the SGs was observed in the secretory acini and proximal portion of the Icds of both goats and sheep but not in mice. This mode of secretion was reported in the PGs of most ruminant species^{17-19,21}. On the other hand, in this study exocytosis of the SGs was observed in the mouse PG, and it has been reported for the SGs with the less electron dense matrix in sheep²¹. Furthermore, the apocrine mode of secretion was not reported in nonruminant animals such as dogs¹⁷, rabbits¹⁵, and horses¹⁶. Moreover, in the horse parotid gland, the SGs reported to extrude from the cell were of the merocrine type¹⁶. Thus, the apocrine mode of secretion may be typical for ruminants but its function is not clear.

The acinar epithelium of the goat PG is reported to be composed of light cells and basal light cells. The light cells contain dense

granules, moderately dense granules and less dense secretory granules. The basal light cells are limited to the basal portion of the acinus, showing no morphological evidence of secretory activity¹⁷. Moreover, it was also reported that the acinus of the PG was composed of three principal cell types: light cells, dark cells and specific light cells, in dogs¹⁷, rabbits¹⁵, horses¹⁶, and bovines¹⁸. The light cells and dark cells contain SGs with high density, moderate density, and low density, together with round corpuscles with high density. The specific light cells contain no secretory granules and their cytoplasmic organelles are sparser than those of light and dark cells. On the other hand, the present study suggested that the variations observed in the cytology and ultrastructure of secretory cells were related to the phases of the maturation cycle or activity (storage, secretory and exhaustion phases) rather than to the existence of different types of cells. The shapes of nuclei and the patterns of their chromatin contents clearly varied with the cell cycle.

The striated duct in the human PG contains some SGs. Mecs are observed in association with both the acinar intercalated and striated duct systems⁴. In the present study, the Mecs were intimately associated with the presence of SGs and apocrine-like protrusions, and Mecs were only observed around the acini and on the acinar sides of intercalated ducts.

In conclusion, the structure of PG cells in goats and sheep, especially the ultrastructures such as the well-developed basolateral membrane infoldings, IcC and microvilli, as well as the presence of numerous secondary lysosomes, are suggestive of copious secretion of saliva with a low protein concentration. On the other hand, the less-developed membrane specialization observed in the mouse PG, as well as the numerous SGs with no signs of lysosomal degradation are suggestive of less saliva secretion with a higher protein concentration. Moreover, the presence of SGs as well as Mecs

in association with the acini and acinar side of the Icd in the goat PG is suggestive of their secretory role, and the role of the other ducts is mainly modification of the primary saliva with no secretory activity. This study also revealed constituent differences in parotid cell ultrastructure compared to other ruminant species depending on the type of diet. Thus the structure of the PG of goats may be intermediate between those reported for the other two feeding types (CS and GR)¹⁶⁻¹⁹. However, more extensive observations are needed to determine whether this is also valid for young goats that depend mainly on milk feeding rather than roughage and concentrate feeding.

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