Original Article

Ultrastructure of the Parotid Salivary Gland in the Greater Cane Rats (*Thryonomys Swinderianus*)

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Abstract

The parotid glands of adult male African greater cane rat (*Thryonomys swinderianus*) were examined by light microscopy (semi-thin sections) and transmission electron microscopy. Histologically, it consisted of acinar cells with vacuoles which corresponded to large oval electron-dense granules, intercalated, striated ducts, and myoepithelial cells which contacted the cells and intercalated ducts (IDs). The cytoplasmic organizations of acinar cells represented the features of serous secreting cells. Ultrastructurally, the acinar cells contained granules of low and moderately electron densities without substructures in their matrix. Lipid droplets were interspersed with the granules. Several coalesced low electron-dense granules were common in some of the acinar cells. The acinar cells also contained few dilated (vesicular) and abundant parallel arrays of tubular rough endoplasmic reticulum and extensive Golgi complex. IDs were lined by tall cuboidal cells interconnected by tight junctions. Secretory granules were absent in their cytoplasm. Striated ducts were composed of columnar cells with few basal cells, and secretory granules were absent as well. Apical blebbing was observed in these ducts. Myoepithelial cells were limited to the acinar-intercalated ductal system. Nerve terminals were observed among the adjacent acinar cells and the underlying basement lamina. The functional significance of these structures is discussed.

Keywords: Cane rats, histology, parotid gland, ultrastructure

Introduction

Salivary glands develop at different sites during embryogenesis and show different histological features with the production of different types of saliva which largely depend on the type of diet.[1] The parotid gland is one of the major salivary glands present in most mammalian species. Salivary glands provide lubrication for eating and vocalization. Salivary secretions of enzymes initiate the digestion of carbohydrates. It also controls bacterial flora through the secretion of lysozyme.[2] Some experiments have demonstrated that it is involved in immunological response through IgA immunoglobulin. It also secretes potassium and reabsorbs sodium.[3] The salivary glands of rodent are important with regard to adaptations to diet, environments, and taxonomic studies.[4]

There is a large volume of literature on the histology and ultrastructure of the mammalian salivary glands including the parotid gland. Extensive reviews of these studies have been done in the various structural aspects of salivary gland in several mammalian species including histology,[5,6] serous cells,[7] mucous cells,[8] myoepithelium,[9] intercalated ducts (ID),[10] striated ducts,[11] and tight junction of secretory cells.[12] In addition, a considerable diversity in the structure of major salivary glands of different species of mammals has been observed earlier at both light and electron microscopic levels.[7,13]

A previous study was on the comparative histology and histochemistry of salivary glands in the African greater cane rat (GCR) (*Thryonomys swinderianus*) and African giant rats (*Cricetomys gambianus*).[14] To the best of our knowledge, there is no description of the ultrastructure of the parotid salivary glands of the GCR in the available literature.

The cane rats also known as grasscutter is a wild herbivorous rodent. It belongs to the suborder *Hystricomorpha* because

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Access this article online

Quick Response Code:

Website: http://www.jmau.org/

DOI: 10.4103/JMAU.JMAU_6_18

How to cite this article: Igbokwe CO. Ultrastructure of the parotid salivary gland in the greater cane rats (*Thryonomys Swinderianus*). J Microsc Ultrastruct 2018;6:17-22.

they have their medial master muscles spread through the infraorbital foramen, whereas the lateral masseter muscles are attached to the zygomatic arches as in primitive rodents.[15] It is related to the African porcupine, brushtail porcupine, guinea pig, chinchilla, and the capybara of South America.[16] It feeds mainly on grass and is capable of digesting majority of edible plants similar to rabbits.[17] It provides a good source of animal protein to many city and urban human dwellers in West Africa and is currently undergoing intensive domestication and captive rearing in parts of Africa.[18] Cane rats can grow up to 13 kg and are among the largest rodents species.[19] Approximately 80 million cane rats are hunted per year in Western Africa, with an equivalent of 300,000 metric tons of meat. In spite of this high level of exploitation, cane rats are by no means endangered, and the numbers are actually increasing in Africa.[20] The species is desired for domestication since, in comparison with most small livestock species, it is culturally better accepted, has a greater carcass yield (ca. 65%) and a superior nutritional value.[19]

The present investigation was undertaken to study the morphology of the normal adult parotid glands of the GCR with emphasis on the ultrastructure of acinar cells, secretory granules, and intralobular ducts. It will also discuss the morphological difference of the gland in this animal from that in other rodents reported previously. The findings will provide useful information that will impact on breeding programs and diagnosis of pathological conditions of the parotid gland. The information obtained will also be important in phylogenetically based interspecies morphological comparisons.

Materials and Methods

Experimental animals

Six[6] adult GCRs (*T. swinderianus*) (2 males and 4 females) that weighed between 6 and 8 kg were used. They were captured by live trapping from the bushes and farmlands around the University of Nigeria, Nsukka, Nigeria and were maintained for 1 week, fed elephant grass (*Pennisetum purpureum*), buffalo grass (*Panicum maximum var trichoghime*), and water *ad libitum*. All the rats were apparently in good health condition before the onset of the experiment. Humane handling of the experimental animals followed the approved guidelines of the Research Ethics Committee Guidelines (2005) of the University of Nigeria.

Experimental procedure

The animals were first given a combination of xylazine hydrochloride (1 mg/kg body weight) and ketamine hydrochloride (15 mg/kg body weight). After 10 min of adequate restraint, they were euthanized by intraperitoneal overdose of thiopental sodium 20 mg/kg body weight (Rox Medica, Germany). Following death, the parotid gland was carefully dissected out, and small pieces of the organ were diced into 1 mm^3 cubes and fixed in 2.5% glutaraldehyde in 0.12 M Millonig's phosphate buffer at pH 7.4. They were postfixed in 1% osmium tetroxide after rinsing in phosphate buffer for electron microscopy. The fixed pieces of the parotid gland were dehydrated in graded ethanol, cleared in propylene oxide, and embedded in epoxy resin. Semi-thin sections (1 μm) were cut and stained with 1% toluidine blue for light microscopy and proper orientation of the tissue blocks. Ultrathin sections (60–80 nm) obtained with Reicher Ultracut® were collected on copper grids, stained with uranyl acetate, and counterstained with Reynold's lead citrate. They were examined under Philips CM10 transmission electron microscope accelerating at 80 KV (FEI, Eindhoven, The Netherlands) with digital camera attachment.

Results

The parotid glands were observed to be purely serous acinar type histologically. The parenchyma consisted of acinar cells and the duct system made up of intercalated and striated ducts [Figure 1]. The acinar cells varied from cuboidal to low columnar with scalloped luminal shape [Figure 2]. Generally, two types of serous cells were apparent in the acini ultrastructurally; (1) predominant typical serous cells with many apical and centrally located secretory granules. These granules were of low and moderate electron densities. Abundant parallel arrays of tubular rough endoplasmic reticulum (RER) and extensive Golgi complex were also present in these acinar cells [Figure 3]; and (2) few light serous cells that contained several dilated, oval vesicular type of rough RER. Large coalesced electro-lucent and moderately electron dense granules were also observed in the second type of secretory cells [Figure 4]. The ducts consisted of intralobular ducts (intercalated and striated), and they coalesced in some parts of the gland to form larger irregular interlobular ducts. Excretory ducts were rarely seen in sections.

Acinar serous cells

The predominant typical serous cells showed basally located large irregular euchromatic nucleus, prominent nucleoli, and highly developed regularly arranged RER. Profiles of short and elongated mitochondria were positioned in between adjacent RER cisternae and also between secretory granules. Fusion of lysosomes and dense secretory granules in the form of phagocytic bodies was apparent in the cytoplasm. Mitochondria were generally few and randomly distributed in the cytoplasm. The basal portion of the acinar cells rested on a prominent basal lamina with smooth contour. Junctional complex was observed between the two adjacent cells. The lateral borders of the cells showed prominent folds with intercellular canaliculi which contained some short cytoplasmic processes of plasma membrane that resembled microvilli [Figure 5]. Axons of nerve terminals were prominent at the basal part of the cell. Myoepithelial cells were observed as long projections between the basal lamina and plasma membrane of acinar cells. They were connected to the acinar cells' desmosomes. They were distinguished by the presence of numerous tightly packed filaments.

The RER was concentrated mostly at the base of the secretory cell and was arranged as parallel rows of cisternae that

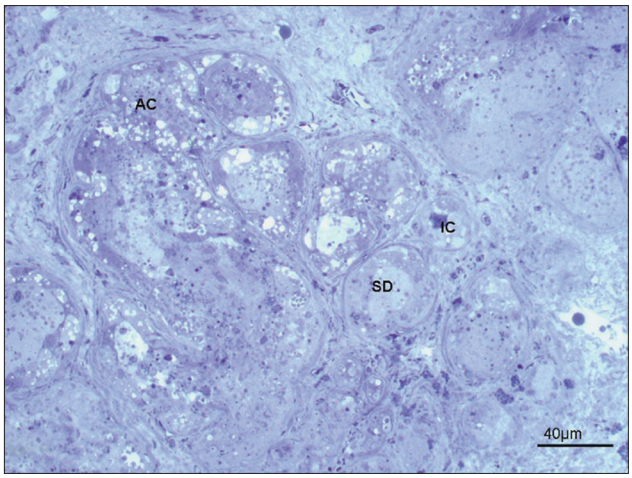

Figure 1: Photomicrograph of semi-thin histological sections of parotid showing an overview of structural arrangement of acinar cells (AC), intercalated duct (IC), and striated duct (SD) within the parenchyma

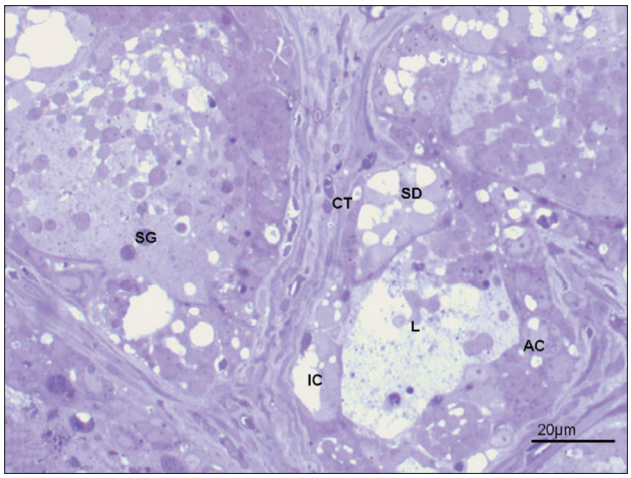

Figure 2: Photomicrograph showing acinar cells with cudoidal cells (AC) filled with secretory granules (SG) and some granules were extruded into the lumen (l). Note the intercalated ducts (IC) that opened into larger striated ducts with intervening connective tissue (CT). Toludine blue stain

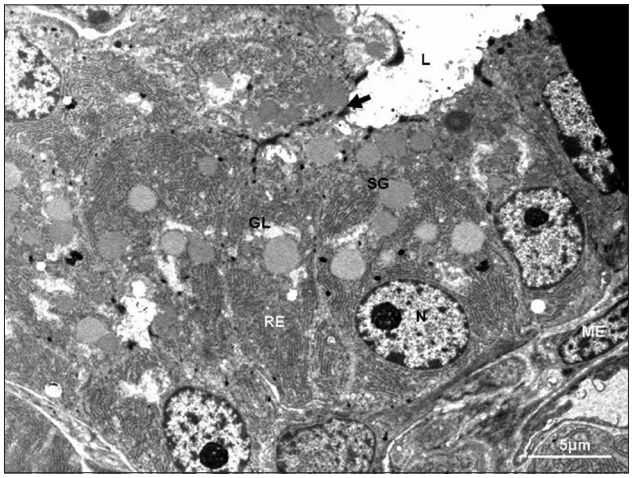

Figure 3: Electron micrograph showing some acinar cells-N (first type) with moderate electron dense granules (SG) and arrays of rough endoplasmic reticulum (RE), Note Golgi complex (GL), lumen of acinus (l) and junctional complex (arrow)

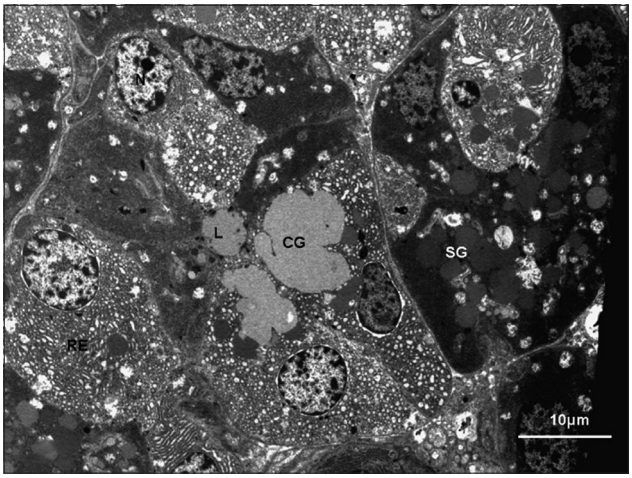

Figure 4: Electron micrograph showing some acinar cells-N (second type) with dilated and vesicular rough endoplasmic reticulum (RE), low dense granules (CG), and lumen of acinus (l)

branched. A common feature of this cell was the presence of one or two oval-shaped lipid droplets among the secretory granules. Some of the secretory granules were extruded from the cell [Figure 6]. Few short stubby microvilli extended from the cells into the lumen, especially in the dark cells with vesiculated RER and also into the intercellular canaliculi.

A prominent feature of the acinar cells was the presence of spherical secretory granules that were of low and moderate electron densities in both types of serous cells. They did not display any substructure in the core (matrix). The limiting membranes of these secretory granules were not conspicuous, and many of them fused with one another. Small pleomorphic electro-dense lysosome-like bodies were seen in the basal and apical cytoplasm of the cells in association with secretory granules. Golgi complexes were sparse. Other cytoplasmic components included occasional lipofuscin.

The few light serous cells (second type) in addition to their short dilated and profiles of parallel arrays of RER also displayed large coalesced electron-lucent granules among profiles of moderately electron-dense secretory granules. Mitochondria were very sparse and the apical cytoplasm of the cells possessed long microvilli which projected into the lumen. Other cytoplasmic components were similar to the predominant serous cell described earlier.

Intercalated ducts

They were infrequently encountered in semi-thin light microscopic sections. The segments were short and inconspicuous. Ultrastrucurally, they were lined by tall cuboidal and even columnar, and these cells were interconnected by tight junctions near an irregular lumen [Figure 7]. Secretory granules were absent in the cytoplasm. Mitochondria were more in the middle part of the cytoplasm around the nucleus and were generally less numerous than in the striated ducts. Golgi

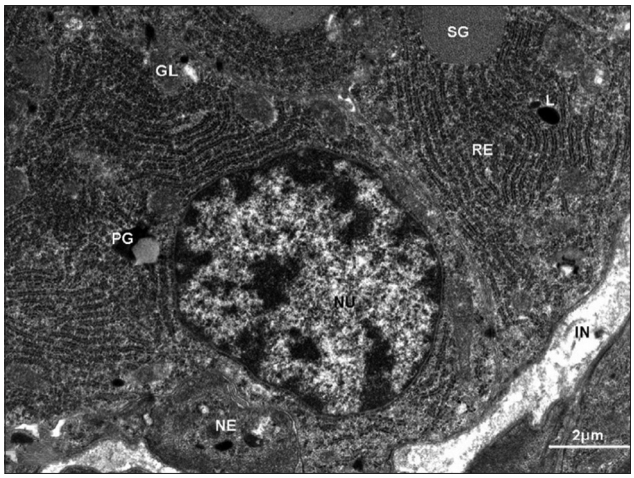

Figure 5: Electron micrograph showing serous cells with highly developed parallel arrays of RER (RE), Golgi complex (GL), secretory granules, lysosomal activity (PG). Note the axons of nerve terminals (NE), and intercellular canaliculi (IN)

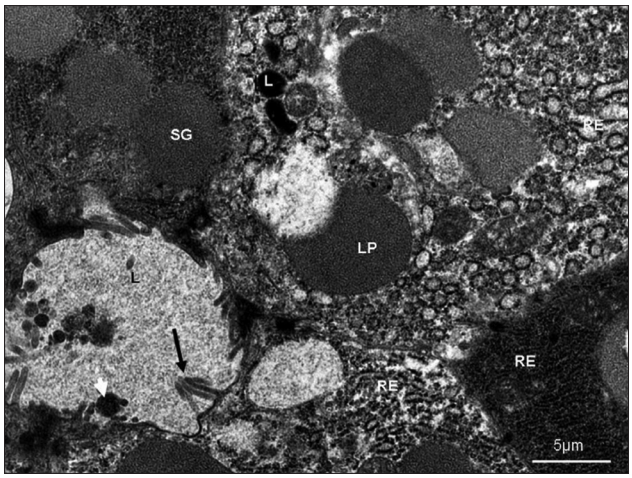

Figure 6: Electron micrograph showing the second type of serous acinar cells with dilated, vesicular rough endoplasmic reticulum (RE), with microvilli (arrow) abutting the lumen (l). Secretory granules (white arrow head) were being taking into lumen Small dense lysosomal bodies (l) are apparent

apparatus was occasionally encountered. Profiles of RER were rarely seen. Numerous diffusely distributed polyribosomes were apparent. Nucleus was centrally located, euchromatic, and contained dense nucleolus. Their apical cytoplasm projected into the lumen as few small bleb-like extensions. Myoepithelial cells and processes enveloped the intercalated ductal cells. They were located between the basal lamina and ductal epithelium and were connected to one another and to the ductal cells by desmosomes.

Striated ducts

They were located intralobularly and were composed of many columnar cells with few basal cells [Figure 8]. The basally situated profiles of mitochondria were interspersed with slightly folded membranes. The predominant columnar cells displayed some vertically arranged mitochondria that resembled the typical basal striations generally ascribed to the cells in this duct. Most of the mitochondria were short slender rods. There were no specific inclusions in the subluminal compartment. The supranuclear region contained a fairly large number of small mitochondria. The apical surface was either smooth or possessed some short microvillous processes. Apical blebbing was observed in some striated ducts, especially at the transitional border into IDs. Myoepithelial cells at the basal membrane of the duct processes were occasionally observed. Nerve terminals were present among the acinar cells and between the acinar cells and the underlying basement membrane.

Discussion

The parotid salivary gland of the GCRs is classified as serous based on the morphological observation of the present study. This opinion is supported by the nature of the secretory granules, which was without a substructure and showed abundant intercellular canaliculi between the glandular serous cells of some mammals including the musk shrew (*Suncus murinus*) according to the findings of Sakai's study.[21] In addition, the secretory cells showed an extensive infranuclear RER that consisted of numerous parallel cisternae as described in some mammals by Tandler and Phillips.[7] The presence of electron-dense granules, extensive RER, and prominent Golgi complexes are features of protein-secreting cells such as the acinar cells of many parotid glands.[5] The earlier report of serous acini on this rodent using light microscopy[14] supports our present investigation. Few stubby microvilli and prominent intercellular spaces were also seen in this study. However, the intralobar ducts did not display any remarkable features different from those of other rodents that have been studied by some investigators.[10,11]

The secretory granules in the present report were moderately electron dense and somewhat heterogeneous and without internal structures (substructures). It is similar to the acini of parotid and Von Ebner's gland of the rat.[5] Mature granules of the rabbit parotid acini are also electron dense but homogeneous. Generally, serous granules show variegated appearance in different mammals and there appear to an evolutionary progression.[7] The simplest form has been observed in the rabbit parotid.[22] They display a uniform dense core, without a trace of substructure or inclusions similar to the present observations in the parotid of the GCRs. However, the serous granules were of moderately electron-dense consistency in most sections examined, whereas a strong electron density of the granules has been observed in many mammals including some rodents.[5] The differences in the nature of secretory granules may be related to diet, circadian rhythms, state of feeding, and mode of fixation for electron microscopy. Even in salivary glands that appear structureless, minor variations in the mode of fixation may show a concealed structure as has been observed in the submandibular gland of the vampire bat.[23] Secretory granules have been shown in their matrix to make the use of laminae, lucent or dense tubules, haloes of

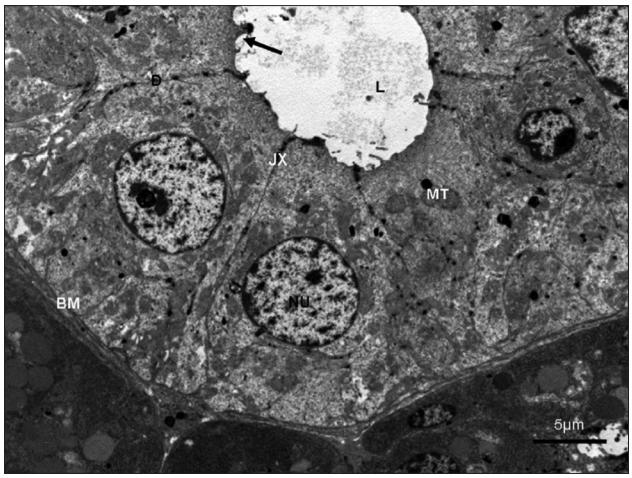

Figure 7: Electron micrograph showing intercalated with high cuboidal cells with euchromatic nucleus (NU), tight junctions (JX), lumen (L), mitochondria (MT). Note the bleb-like extensions (*arrow*) of the apical cytoplasm. Basement membrane and desmosomes (D) are apparent

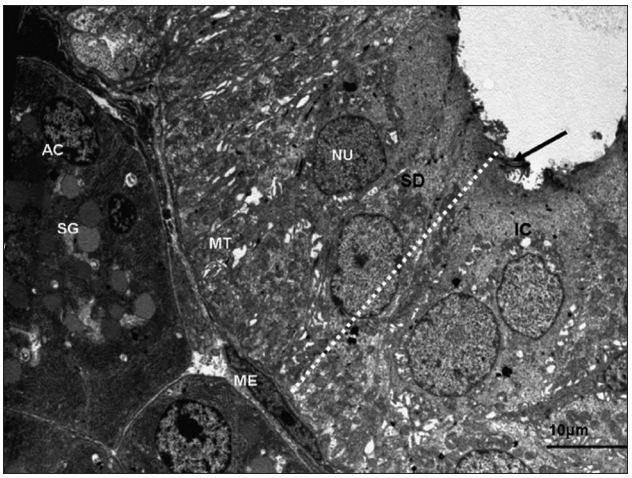

Figure 8: Electron micrograph of striated duct at the transitional border (*dotted line*) with intercalated duct (IC). Note the columnar cells (NU) of the striated duct with copious basally located mitochondria (MT). Some myoepithelial cells (ME) are present. The adjacent acinar cells (AC) with secretory granules are represented

both dark and light, punctuate densities, crystalloids, filaments, and structureless matrix.[7] Many investigators have shown that fixation may markedly alter secretory granules as do administration of exogenous substances.[5] secretory granules of many salivary glands contain proteins (both enzymatic and nonenzymatic), polysaccharides, and electrolytes, and these apparently sort themselves out according to chemical affinities, charge, steric effects to yield specific patterns seen in electromicrocopy according to species.[24] Therefore, further studies will involve using different fixatives for electron microscope and immunohistology with emphasis on the nature of secretory granules and the secretory process.

Few short microvilli were observed on the apical plasma membrane of the secretory cells in the present investigation as in several rodents.[5] The precise function of apical microvilli on serous cells is not yet known precisely, but it is likely that they increase the absorptive surfaces of the acinar or tubular lumina, just as microvilli do on the thyroid cells and epithelial cells. They may, therefore, help in the concentration of the primary saliva by removal of water.[25] In addition, the serous acini in the present study were made up of epithelial cells surrounded by distinct basement membrane. Basal striations of the plasma membrane were observed as have been elaborately described in many mammalian parotid glands and submandibular salivary serous or seromucous gland.[23,26]

Prominent intercellular spaces with few processes were observed between the serous cells in the present study as have been described in salivary glands of several mammals. Intercellular spaces seen in this work resembled those that occur between epithelial cells lining the gallbladder of several species. It is believed that the lateral intercellular spaces play a major role in the transepithelial movement of water.[25]

Lipid droplets observed in the acinar cells in the study may be as a result of fluid intake, starvation, or fasting before the commencement of the present experiment. Lipid droplets have been shown to occur in serous and mucous cells of parotid and submandibular gland of albino rats under experimentally high-fat diet-induced hyperlipidemic rats seen as intracellular droplets.[27] Marked accumulation of lipid droplets in parotid is also observed after administration of some substances such as actinomycin, alloxan, and isopreterenol.[5] The myoepithelial cells encountered in basal lamina of serous acini and in association with ID cells' function in contraction, when the gland is stimulated to secrete, compressing or stabilizing the underlying parenchymal cells, thus helping in the expulsion of saliva. It also helps in preventing damage to other cells through this process. They may also aid in the propagation of secretion or other stimuli.[9]

ID with its typical simple squamous epithelium was observed in the parenchyma and constituted a negligible percentage of the parenchyma as in the rat (4%), rabbit (2.8%), and several other rodents.[10] Secretory granules are normally difficult to ascertain in the IDs of many species[5] and were not observed in the present study. Similarly, it was not observed in the parotid of Mongolian gerbil (*Meriones meridianus),* South American field mouse (*Mus minutoides*), and African ground squirrel.[10,28] Granules of ID are present in the parotid of some mammals including the nine-banded armadillo, common rabbit, and several bat species.[10] RER was sparse, except for few scattered cisternae in the intercalated ductal cells. This is in contrast to the ductal cells of parotid of mouse and "four-eyed" opossum,[10] which contain a substantial development of RER. IDs convey saliva to the larger intralobular ducts. It also modifies the saliva by adding assortment of glycogenates to it and equally influences the electrolyte composition of the saliva.[11] The presence of nerve axon terminals in the acini and close to the basement membrane may provide structural evidence of a quick response of the parotid acinar cells during stimulation. The phagocytic activity of lysosomal bodies in the cytoplasm of acinar cells functions in the regulation of

the secretory process by providing the mechanism which influences the overproduction of secretory products.

It could, therefore, be concluded from this study that the histological and ultrastructural features of the parotid gland of the GCRs do not differ significantly from that of other rodents such as hamsters, rats, mouse, rabbits, and Mongolian gerbils except in the absence of substructures in the matrix of secretory granules of the acinar cells which may be due to fixation techniques, diet, or the environment.

Acknowledgement

The author which to thank Erna Van Wilpe of the electron microscopic unit, Fcaulty of Veterinary Science, University of Pretoria, for her laboratory care, and Prof D. N. Ezeasor, for showing the academic way of doing it.

Financial support and sponsorship

This research was not funded by any agency but necessary basic facilities were provided by the University of Nigeria**.**

Conflicts of interest

There are no conflicts of interest.

References

1. Jaskoll T, Zhou YM, Chai Y, Makarenkova HP, Collinson JM, West JD, *et al.* Embryonic submandibular gland morphogenesis: Stage-specific protein localization of FGFs, BMPs, pax6 and pax9 in normal mice and abnormal SMG phenotypes in fgfR2-IIIc(+/Delta), BMP7(-/-) and pax6(-/-) mice. Cells Tissues Organs 2002;170:83-98.
2. Genkins GN. In: Physiology and Biochemistry of the Mouth. 4th ed. Oxford: Blackwell Scientific; 1978. p. 284-359.
3. Ferraris ME, Arriaga A, Busso C, Carranza M. Histological study of parotid, submaxillary and von ebner salivary glands in chronic alcoholics. Acta Odontol Latinoam 1999;12:97-102.
4. Stimson RH, Johnstone AM, Homer NZ, Wake DJ, Morton NM, Andrew R, *et al.* Dietary macronutrient content alters cortisol metabolism independently of body weight changes in obese men. J Clin Endocrinol Metab 2007;92:4480-4.
5. Pinkstaff CA. The cytology of salivary glands. Int Rev Cytol 1980;63:141-261.
6. Amano O, Mizobe K, Bando Y, Sakiyama K. Anatomy and histology of rodent and human major salivary glands:-Overview of the Japan salivary gland society-sponsored workshop-. Acta Histochem Cytochem 2012;45:241-50.
7. Tandler B, Phillips CJ. Structure of serous cells in salivary glands. Microsc Res Tech 1993;26:32-48.
8. Tandler B. Structure of mucous cells in salivary glands. Microsc Res Tech 1993;26:49-56.
9. Redman RS. Myoepithelium of salivary glands. Microsc Res Tech 1994;27:25-45.
10. Tandler B, Nagato T, Toyoshima K, Phillips CJ. Comparative ultrastructure of intercalated ducts in major salivary glands: A review. Anat Rec 1998;252:64-91.
11. Tandler B, Gresik EW, Nagato T, Phillips CJ. Secretion by striated ducts of mammalian major salivary glands: Review from an ultrastructural, functional, and evolutionary perspective. Anat Rec 2001;264:121-45.
12. Baker OJ. Tight junctions in salivary epithelium. J Biomed Biotechnol 2010;2010:278948.
13. Young JA, Van Lennep EW. Morphology of Salivary Glands. 1st ed. London: Academic Press Inc.; 1978. p. 72-108.
14. Igbokwe CO, Neba PC, Bello UM. Comparative histologty and histochemistry of major salivary glands in the giant pouched-rats (*Cricetomys gambianus*) and greater cane rats (*Thryonomys swinderianus*). Indian J Anim Res 2015;49:451-60.
15. Allaby M. Hystricomorph: A Dictionary of Zoology. Oxford: Oxford University Press; 1999.
16. National Research Council. Microlivestock: Little Known Small Animals with Promising Economic Future. Washington DC, USA: National Academy Press; 1991. p. 17-449.
17. Uwalaka RE, Ahaotu EO. Perfomance of growing grasscutters on different fibre sources. Int J Vet Sci 2013;2:85-7.
18. Asibey EO, Addo PG. The grasscutter, a promising animal for meat production. In: Turham D, editor. African Perspectives: Practices and Policies Supporting Sustainable Development. Harare, Zimbabwe: Scandinavian Seminar College, Denmark, In Association with Weaver Press; 2000. p. 120.
19. Odebode A, Awe F, Fumuyide OO, Adebayo O, Ojo B, Daniel G. Households consumption patterns of grasscutter (*Thryonomys swinderianus*) meat within Ibadan metropolis, Oyo State, Nigeria. Cont J Food Sci Technol 2011;5:49-57.
20. Jori F, Lopez-Beajar M, Houben P. The biology and use of the African brush-tailed porcupine (*Atherurus africanus*, Gray, 1842) as food animal. A review. Biodivers Consev 1998;7:1417-26.
21. Sakai T. Major ocular glands (harderian gland and lacrimal gland) of the musk shrew (*Suncus murinus*) with a review on the comparative anatomy and histology of the mammalian lacrimal glands. J Morphol 1989;201:39-57.
22. Castle JD, Jamieson JD, Palade GE. Radioautographic analysis of the secretory process in the parotid acinar cell of the rabbit. J Cell Biol 1972;53:290-311.
23. Tandler B, Toyoshima K, Phillips CJ. Ultrastructure of the principal and accessory submandibular glands of the common vampire bat. Am J Anat 1990;189:303-15.
24. Tandler B, Nagato T, Philips CJ. Systematic implications of comparative ultrastructure in the subamandibular salivary gland of aribeus (*Chiroptera phyllostomidae*) J Mammol 1986;67:81-90.
25. Pinkstaff CA. Cytology, histology and histochemistry of salivary glands; An overview. In: Vergona D, editor. Biology of Salivary Glands. Boca Raton, FL: CRC Press; 1993. p. 55-38.
26. Riva A, Lantini MS, Testa F. Normal human salivary glands In: Riva A, Motta PM, editors. Ultrastrucuture of the Extraparietal Glands of the Digestive Tract. Boston: Kluwer Academic Publishers; 1990. p. 53-74.
27. Kim SK, Allen ED. Structural and functional changes in salivary glands during aging. Microsc Res Tech 1994;28:243-53.
28. Ichikawa M, Ichikawa A. The fine structure of the parotid gland of the Mongolian gerbil, *Meriones meridianus*. Arch Histol Jpn 1975;38:1-6.