Comparative histology and histochemistry of the major salivary glands in the giant pouched-rats (*Cricetomys gambianus*) and greater cane rats (*Thryonomys swinderianus*)

Igbokwe Casmir Onwuaso*, Chebe Peter Neba and Umar Muhammed Bello

Department of Veterinary Anatomy,
Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.

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**ABSTRACT**

The comparative morphometry, histology and histochemistry of the submandibular, parotid and sublingual glands were studied in the wild adult African giant pouched rat (*Cricetomys gambianus*) and greater cane rats (*Thryonomys swinderianus*). The submandibular was purely serous in the giant pouched rats, but sero-mucous in the cane rats with serous acini predominating. Serous demilunes were common in the submandibular gland of cane rats but not in that of giant rats. The parotid contained serous acini in both species, in addition intermingled copious globular fat tissue was observed in the cane rats. The sublingual gland showed sero-mucous acini with mucous predominating in both rodents. Large striated (intralobular) ducts were present in the parenchyma of both rats. Strong activity of neutral mucins was observed in submandibular and parotid gland of both rodents, however acidic mucins were absent. The sublingual gland showed reactivity for neutral and acidic mucins in both rats. Gross morphometry indicated significant differences (p<0.05) in the weights of the three glands in between the rodents. Micrometry showed significant differences between the rats in the diameter of striated ducts of submandibular gland, but such significant differences did not exist in the striated ducts of parotid and sublingual glands.

**Key words:** Histochemistry, Histology, Major salivary glands, Rats.

**INTRODUCTION**

Mammals possess well developed major salivary glands (submandibular, parotid, sublingual and zygomatic glands). The glands develop from ectodermal lining of the primitive oral cavity (Poddar and Jacob, 1977; McGeady et al., 2006). Salivary glands provide lubrication for eating and vocalisation. Salivary secretions of enzymes initiate the digestion of carbohydrates. It also controls bacterial flora through the secretion of lysozyme (Genkins, 1978). Some experiments have demonstrated that it is involved in immunological response through IgA immunoglobulin. It also secretes potassium and reabsorbs sodium (Ferraris et al., 1999). Specifically, the salivary glands of rodent are important with regard to adaptations to diets, environments and taxonomic studies (Stimson et al., 2007). Variations in production of saliva can be affected by numerous physiological and pathological conditions. Salivary secretions are usually divided into two categories, serous and mucous. Serous secretion is thin and watery and consists of water, inorganic ions and some proteinaceous material, while mucous secretions is more viscous and contained a higher concentration of organic proteinaceous matter including neutral and acidic glycoproteins (Schneyer and Schneyer, 1967).

The African giant pouched-rats (GPR) (*Cricetomys gambianus*) belong to the family –*Nesomyidae*. They are nocturnal rodents and native to sub-saharan Africa. The adult males weigh about 1-1.5 kg. They are omnivorous rats and feed on vegetation, palm fruits, palm kennels, bread (grain products) and invertebrates such as insects. They are partly fossorial rodents, living in burrows with several tunnels and outlets and come out at night to briskly search for food particularly around domestic homes and sewage systems. These burrows have been reported in some rodents to have low oxygen concentration and high carbon dioxide concentration (Chapman and Bennet, 1975). Extreme hypoxia and hypercapnia affects cardiac function in most mammals. This may in turn affect the secretions of the salivary glands into the oral cavity (Tucker et al., 1976).

The Greater cane rats (GCR) (*Thryonomys swinderianus*) also known as Grasscutter, belong to the sub-
order- Hystricognathi and family-Thryonomydae. It is a wild herbivorous rodent, that feeds on shoot and stem of grass and reeds that grow in damp places (Skinner and Smither, 1990). In Southern Africa, it is particularly associated with cane fields (NRC, 1991). They are nocturnal rodents and make nests from grasses or live in shallow burrows underground. The typical adult weight is about 5-8 kg.

The GPR and GCR provide supplementary dietary protein supply to some rural dwellers in West Africa. GPR have potential for use as laboratory animal (Adu et al., 2005) and have been utilized in Southern Africa for sniffing of land mines (Lindow, 2001), and diagnosis of tuberculosis (Poling et al., 2010). Both rodents are currently undergoing intensive domestication and captive rearing in farms across parts of West Africa for several purposes that include biomedical research.

Abundant information on salivary glands exist for several laboratory rodents, human-beings and domestic animals (Pinkaff, 1980 for review; Espinal et al., 1983; Watanabe et al., 1997; Khojasteh and Delashoub, 2012), but there is little information on other rodents that are found in the African tropical region, particularly the giant pouched-rat and greater cane rats.

Even so, the existing information on histochemistry and morphometry of these salivary glands in the giant pouched-rats is quite conflicting (Pinkaff, 1980; Asojo and Aire, 1983; Ikpegbu et al., 2013).

This work is aimed at examining the histological and histochemical features of the submandibular, parotid and sublingual gland and relating it to their diet. The information provided will be useful in dietary management of the animals in captivity and clinical diagnosis of diseases of salivary glands. Comparative studies of salivary glands will help to gain insight into the morphological and functional diversity within mammalian cell types.

MATERIALS AND METHODS
Experimental animals: Ten (10) adult African giant pouched-rats (4 males and 6 females) used in the study were captured from the wild bushes around the University town of Nsukka and maintained for one week in the laboratory animal house (Veterinary Anatomy Dept, UNN) and were fed ad libitum with palm nuts, bread, root tubers (yam and cassava), paw-paw, groundnut, commercially prepared animal feed and clean drinking water were also provided. Also, six (6) adult cane rats (2males and 4 females) used were captured by live-trapping from the bushes mentioned above from and were maintained for one week, fed elephant grass (pennisetum purpureum), buffalo grass (Panicum maximum var trichoghime) and water ad libitum. They were all 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 giant rats and cane rats were euthanized by intraperitoneal overdose of Thiopental sodium 20mg/kg body weight (Rox Medica, Germany). Following death, the animals were weighed. The salivary glands were also carefully dissected out and weighed with a digital weighing balance. Cut blocks of salivary gland tissue (submandibular, parotid and sublingual) were fixed in 10% neutral buffered formalin (NBF) solution for 36-48 hours and dehydrated in graded concentrations of ethanol (70%, 80%, 90%, and 100%). Tissues were cleared in xylene and embedded in paraffin blocks and sections of 5µm thickness were cut with a Leitz® rotary microtome and mounted on glass slides. Sections were stained with haematoxylin and eosin (H&E), periodic acid Schiff reaction (PAS) with diastase digestion as negative controls, and Alcian blue (AB pH 2.5) following routine histological and histochemical procedure (Bancroft and Gamble, 2002). They were examined under a microscope and selected tissue sections were captured into a computer with Moticam 2005 camera attachment (Moticam China).

Statistical analysis: The mean, standard error of mean (SEM) and range of measured gross and micrometric parameters were determined for each salivary gland in the species using SPSS version 16.0 for Microsoft windows. For micrometry, cross-sectional diameter of oval to round striated duct were measured (x40) using ocular micrometer gauge calibrated with stage micrometer from randomly selected sections. Comparison of data obtained were analyzed using the student’s t-test and significance accepted at p<0.05.

RESULTS AND DISCUSSION
Histological observations: The histological sections of the submandibular of giant pouched-rats (GPR) showed a thin connective tissue capsule that provided septa into the parenchyma with clear lobular divisions. The submandibular gland was tightly related to the corresponding sublingual gland and were enclosed in a common connective tissue capsule with a very thin connective tissue strand that separated them. In the interlobular connective tissue of the gland, blood vessels and nerves were present (Fig. 1). The GPR submandibular gland was purely compound-tubulo-alveolar serous secretory units. The serous cells were lightly basophilic, granular, with rounded or spheroidal nuclei placed more centrally. The ductal system was well developed and included, the intercalated duct with flattened or cuboidal cells,
striated ducts with cuboidal or columnar cells containing basal striations and larger excretory ducts with taller columnar cells situated mainly in interlobular connective tissue (Fig.2). Granular ducts interposed between the intercalated and striated ducts were occasionally seen and consisted of pyramidal cells with basally situated nuclei and basal striations. They contained numerous granules in the apical cytoplasm. Myoepithelial cells with flattened nuclei were sometimes identified on the outer layer of the acinar secretory cells and in the proximal portion of the ductal system, especially intercalated duct. The intralobular duct (striated ducts) contents were PAS negative and reacted moderately to AB. The acini were weak to moderately PAS positive and moderate to strong activity for acid mucopolysaccharides was observed (Fig.3). No mucous acini or demilunes were observed in this gland and was confirmed by histochemical staining properties.

The submandibular gland of the greater cane rats (GCR) in contrast showed predominantly serous acini with few scattered mucous acini in various lobules of the gland (Fig.4). The serous secretory units in addition to the typical features described above possessed many intralobular ducts (intercalated and striated) and larger ducts were commonly observed, which in certain areas coalesced into larger irregular interlobular ducts. Serous demilunes were commonly observed and these demilunes were identified by their strong PAS positive staining. The serous acini were strongly PAS positive and the few intermingled mucous acini were moderately PAS positive. The contents of the numerous intralobular ducts were moderately PAS positive. The serous acini were AB negative (-) and the few scattered mucous acini were strongly AB positive (Fig.5).

The parotid in both species (GPR and GCR) generally consisted of compound tubulo-alveolar units surrounded by a thin capsule of collagenous fibres. The septa were very thin and divided the parenchyma into lobules and
FIG 5: Photomicrograph of GCR submandibular gland showing positive reaction to PAS (A) and intense reaction to AB-arrows (B). Bar scale 20 µm.

FIG 6: Photomicrograph of GPR parotid gland showing well a developed ductal system including striated duct (D), intercalated duct (C) and serous acini (S) with round to oval cells (arrow). H & E. Bar scale 20 µm.

FIG 7: Photomicrograph of parotid gland of GCR showing serous acini (S) with highly distinct ducts and intermingled fat globular tissue (F) in the interlobular and intralobular zones (arrows). H & E. Bar scale 30 µm.

FIG 8: Photomicrograph of histochemical reaction of GPR (A) and GCR (B) parotid gland to PAS and AB (pH 2.5), it showed strong reaction to PAS (arrow) and very weak reaction with AB. Bar scale 20 µm.

FIG 9: Photomicrograph of GPR sublingual gland showing one of the several large intralobular ducts (D), intercalated duct (C), serous demilune caps (C) on mucous acini (M). H & E. Bar scale 20 µm.

contained blood vessels and nerves. In the lobules, the parenchyma were made up of purely serous acini and ducts. Each acinus was comprised of 4-7 pyramidal cells with basal spherical nuclei that enclosed minute lumen. Stellate-shaped myoepithelial cells surrounded the acinus. In both species also, the duct system comprised of intercalated duct, striated duct and intralobular excretory ducts (Fig.6). However several highly elongated interlobular ducts with pseudostratified columnar epithelium were more frequently encountered in the parotid of GPR. Fat globules were remarkably present in the interlobular connective tissue close to the secretory units of the parotid of GCR (Fig.7).

Histochemically, the GPR serous acini were strongly PAS positive and the luminal contents were weakly PAS positive. The acinar cells and the lumen of ducts reacted negatively to AB (pH 2.5) in parotid of the two species (Fig.8).

The sublingual gland in the GPR was apposed to the submandibular and separated by thin connective tissue elements. It comprised of a mixed compound mixed tubuloalveolar secretory units and was predominantly made up of mucous acini with few serous demilune caps. The voluminous acini showed large irregular lumen. The mucous cells possessed a spongy cytoplasm with flattened nuclei located at the base. Intercalated ducts were not frequently observed unlike oval striated ducts with cuboidal epithelium and basal striations were distinct (Fig.9). In addition many large oval or elongated intralobular and interlobular ducts...
excretory ducts lined with stratified cuboidal cells were commonly observed in the parenchyma. Myoepithelial cells were observed in contact with secretory cells and occasionally in association with intercalated ducts. The mucous acini of GPR sublingual gland were moderately PAS ypositive; the serous demilunes were intensely stained with PAS (Fig. 10). The lumen of the ducts was moderately PAS positive. The mucous acini and the luminal contents of all grades of ducts in the gland reacted strongly to AB staining. The sublingual gland of GPR (Fig. 11), showed similar features to that of GPR by the presence of predominantly mucous acini intermingled with grades of oval and large elongated ducts in the intralobular and interlobular tissue. Distinct striated ducts were rarely observed in the sublingual gland of both species. Similarly, the mucous acini reacted strongly to PAS and the luminal contents of the highly elongated ducts were moderately PAS positive. It reacted strongly to AB in the mucous acini and luminal contents of the highly elongated irregular ducts (Fig. 12).

Gross morphometry showed significant differences (p<0.05) in measured weights of the three types of salivary glands in the two rodents (Table 1). Micrometrically significant differences were observed between the two rodents in relation to the measured parameters of the submandibular gland (p<0.05 (Table 2). However there were obvious similarities in size of measured ducts in the parotid and sublingual glands of the two rodents (Table 3 and 4).

The histology, histochemistry and ultrastructure of salivary glands are known to show great diversity amongst mammals and quite often sex differences exit within same species. Staining methods for mucosubstances have demonstrated that gross anatomically, comparable glands from different species may secrete entirely different mucosubstances (Pinkaff, 1980; Elewa et al., (2010). The present work demonstrated both histologically and histochemically that the giant pouched-rats (GPR) submandibular gland consisted of purely serous acini secretory unit, while that of the greater cane rat (GCR) was predominantly serous with few intermingled mucous acini. The present observation in GPR is similar to the report by Asijo and Aire (1983), but Ikpegbu et al., (2013a) using only histological method (H &E staining) reported the presence of two compartments in the gland made up of serous and mucous units. To our present knowledge this report appears to be erroneous, because the close apposition of the lobes of the sublingual salivary glands to the submandibular in several rodents including the present species of study may be

<table>
<thead>
<tr>
<th>Animal Species</th>
<th>Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPR</td>
<td>0.573±0.01</td>
</tr>
<tr>
<td>GCR</td>
<td>5.221±0.02</td>
</tr>
</tbody>
</table>

Note: GPR (African giant pouched rat), GCR (greater cane rats). Also means in the same column with different superscript differ significantly at p<0.05.
TABLE 2: Micrometrical parameters relating to the cross-sectional diameter of oval/round–shaped striated duct (SD) of giant pouched-rats and cane rats submandibular glands

<table>
<thead>
<tr>
<th>Species/salivary glands</th>
<th>Diameter of SD (µm)</th>
<th>Epithelia thickness (µm)</th>
<th>luminal diameter of SD (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPR-SM</td>
<td>37.2±1.09 (25.4-40.1)</td>
<td>10.5±1.04 (9.4-12.1)</td>
<td>11.2±1.3 (9.6-12.2)</td>
</tr>
<tr>
<td>GCR-SM</td>
<td>57.1±2.19 (48.1-60.2)</td>
<td>24.7±0.05 (19.5-25.5)</td>
<td>23.8±1.9 (23.4-30.1)</td>
</tr>
</tbody>
</table>

Note: GPR –Giant pouched-rat, GCR- greater cane rats, SM-submandibular gland, SD-oval/round striated ducts. Note that different superscripts that are in the same column do not differ significantly at P<0.05.

TABLE 3: Micrometrical parameters relating to the cross-sectional diameter of oval/round–shaped striated duct (SD) of giant pouched-rats and cane rats parotid gland

<table>
<thead>
<tr>
<th>Species/salivary glands</th>
<th>Diameter of SD (µm)</th>
<th>Epithelia thickness (µm)</th>
<th>luminal diameter of SD (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPR-PT</td>
<td>51.1±2.17 (45.2-54.1)</td>
<td>22.4±2.07 (21.5-24.6)</td>
<td>23.5±1.06 (22.1-24.5)</td>
</tr>
<tr>
<td>GCR-PT</td>
<td>54.1±2.71 (50.2-55.3)</td>
<td>23.5±2.05 (20.2-25.3)</td>
<td>23.7±1.18 (21.7-27.2)</td>
</tr>
</tbody>
</table>

Note: GPR –Giant pouched-rat, GCR- greater cane rat, PT-parotid salivary gland, SD-oval/round striated ducts. Note that similar superscripts that are in the same column do not differ significantly at P<0.05.

TABLE 4: Micrometrical parameters relating to the cross-sectional diameter of oval/round–shaped striated duct (SD) of giant pouched-rats and cane rats sublingual gland

<table>
<thead>
<tr>
<th>Species/salivary glands</th>
<th>Diameter of SD (µm)</th>
<th>Epithelia thickness (µm)</th>
<th>luminal diameter of SD (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPR-SL</td>
<td>48.2±2.31 (45.2-54.1)</td>
<td>25.3±1.01 (21.5-24.6)</td>
<td>21.2±1.16 (22.1-24.5)</td>
</tr>
<tr>
<td>GCR-SL</td>
<td>46.3±2.21 (50.2-55.3)</td>
<td>30.2±1.72 (20.2-25.3)</td>
<td>23.5±1.61 (21.7-27.2)</td>
</tr>
</tbody>
</table>

Note: GPR –Giant pouched-rat, GCR- greater cane rat, SL-sublingual salivary gland, SD-oval/round striated ducts. Note that similar superscripts that are in the same column do not differ significantly at P<0.05.

FIG 12: Photomicrograph of GCR sublingual gland reaction to PAS and AB with numerous ducts. The mucous acini showed moderate reaction to PAS and strong reaction to AB. Note that demilunes caps were not commonly encountered here unlike in that of GPR sublingual gland. Also large ducts with PAS and AB reactive contents were common. Photomicrographs below are larger magnifications of the above. Bar scale 60µm (above) & 20µm (below).
diet and living environments. The presence of well developed serous secretory units might be the need for increased breakdown of solid carbohydrates diet by amylase and lysozyme production (an antibacterial agent) may help to ward off infectious agents in the wild rodents like giant pouched-rats and the greater cane rats involved in the study.

The parotid gland of the species studied showed well developed lobulated gland with typical serous acini and several intermingled intralobulated duct (intercalated and striated ducts) and larger interlobular ducts. These features particularly the purely serous acini is similar to that reported in many rodents and mammals including European hamster (Khojasteh and Delashoub, 2012), giant rats (Asojo and Aire, 1983), African palm squirrel (Ikpegbu et al., 2013b), rabbits (El-Ramli et al., 2013), armadillos (Zedyus pichiy) (Estecondo et al., 2005). It is also serous in the rat, mouse, bank voles but sero-mucous acini are common in field voles (Siuda and Szymanska, 1961). However the parotid of some carnivorous mammals including dog, cat and ferret is reported to be sero-mucous (Poddar and Jacob, 1977). Vignoli and Nogueira (1981) found only serous acini in older zebu cattle (Bos indicus) animals, but mucous acini in younger animals. An increase in dry carbohydrate diet produced a fluid serous secretion, while a case of juicy diet; the salivary glands secrete more mucous (Siuda and Szymanska, 1961). The giant pouched-rats are known to consume a wide variety of hard carbohydrate diet that include palm-kernels, yams and cassava tubers and this might be responsible for the well developed serous acini and ductal system of the parotid gland. On the other hand, the greater cane rats feed basically on grass but the harsh wild environment usually subject these rats to other tougher carbohydrate foods like root tubers, hence the presence of well formed purely serous acini as well and larger excretory ducts. However, some other factors play a role in modifying salivary secretion. Bellavia et al., (1992), has shown the influence of circadian variations in the morphology, physiology and biochemistry of salivary glands. Also Nawar and El-Khaligi (1975) earlier, also demonstrated that in the parotid of camels, the histochemical appearance changes with feeding cycles as it passes from phases of rest to activity and then exhaustion. Serous cells are generally considered to be active in synthesis and secretion of proteinaceous substances and the presence of a high concentration of rough endoplasmic reticulum is often accepted as an indicator of cells capacity for synthesis and secretion of proteins (Pinkaff, 1980). Amylase comprise about 40% of the secretory protein in parotid of the mouse whereas sialomucin is the major secretory product of sublingual gland (Vreudgdeblil et al., 1982).

Copious adipose tissue intermingled with serous parenchyma and sepal connective tissue in the parotid of the cane rats but not in the giant rat. This difference may be because the cane rats unlike the giant rats have larger body size and does not burrow into complex tunnels that require much energy, hence the accumulation of adipose tissues in certain organs like the parotid gland. Similar accumulation of fat droplets has been reported in the acinic cells of aging rat parotid. It has been reported that high-fat diet caused intracellular lipid accumulation in the parotid and sublingual glands of Wistar albino rats (Pisivicilar et al 2009). There are also other factors causing accumulation of lipid droplets in salivary glands such as ingestion of liquid diet, starvation, prolonged hypoxia, diabetes mellitus and ageing amongst others (Kim and Allen, 1994; Anderson et al., 1994). The present finding in greater cane rats might also be related to ageing due to reduced level of cellular secretory activity as has been suggested to be case during atrophy of the gland due to liquid diet (Hand, 1972).

The sublingual gland in giant-pouched rat is similar in both histological and histochemical characteristics to the cane rats as in most mammals (Shackleford, 1963). It was predominantly mucous in the current study as in most rodents, ruminants and swine (Pinkaff, 1980). In both species, well developed striated ducts (intralobular) with only few intercalated ducts were encountered, similar to that of desert rats and antelope squirrel (Shackleford and Schneyer, 1964). Histochemical results in the present study have confirmed the presence of diverse products being produced by different cytological structures within same gland as has been noted extensively by Pinkaff (1993). The current work demonstrated that the submandibular glands of giant pouched-rats and cane rats contained neutral mucins and acidic mucins, but the acidic mucins were only detectable from the mucous acini of the giant rats. The serous acini of the cane rats did not demonstrate acidic mucins. Neutral mucins were detected in the mucous acini of sublingual glands in the two species, while serous demilunes and ducts were devoid of neutral mucins. Acidic mucins were present in the mucous acini and serous demilunes of sublingual glands. These findings do not differ from those of Asojo and Aire (1983) in the giant rats and some observations in other mammals (Shackleford, 1963; Zhang et al., 2005). Neutral mucins were strongly demonstrated in the parotid of giant rats and weakly in the acini of cane rats, while neutral mucins were not detected in the ducts. However acidic mucins were not completely detected in the serous acini of the parotid in the two species. Privy (1986), similarly detected neutral mucins in parotid of guinea pigs and sheep, while that of cats and dogs showed
sulphated acid mucins. The current findings suggest that the differences in the histochemical reaction might be due to varying dietary components, living environment and genetic constitution. It has been shown that submandibular and sublingual glands showed variety of acid mucins (sulphated and sialomucins) in all mammals (Dhabale, 2014). Salivary mucins are important factors in preservation of oral cavity. The large glycoprotein play a major role in formation of protective coating of tooth enamel and oral mucosa, which act as dynamic barrier capable of modulating effects of environment (Taylor and Flaa, 1994), particularly for the wild species used in the current study, that are often exposed to the inclement tropical humid environment.

The cross-sectional diameter of the striated ducts in GPR and GCR in all the salivary glands studied presently varied significantly. In the submandibular gland specifically the values were 37.2 ± .9 μm and 57.19 μm in GPR and GCR respectively. These values were different from obtained in Tien Shan shrew (24.18± .02μm) and Eared- hedgehog (43.96 ± μm) according to Bazarbayeva et al., (2013). Variations in diameter of conducting ducts like the striated duct can be influenced by the size of the rodent, age, sex, diet and even the time of the day of sacrifice. The present work showed extreme species variability in the size of the channels of salivary secretion in rodents.

In conclusion, the major salivary glands in the giant rats and cane rats show significant morphological differences in relation to the submandibular gland. The parotid and sublingual gland showed similar structural and histochemical properties. The current observations did not differ significantly from observations in several mammals including human beings. Therefore the present work has provided baseline data that may be useful in knowing the pathophysiology of diseases of salivary glands, and in biomedical modelling of effects of nutrition on salivary glands of mammals.

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