

HISTOMORPHOLOGY AND HISTOCHEMISTRY OF ADENOMERES OF VENTRAL BUCCAL GLAND IN BUFFALO (*Bubalus bubalis*)

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ABSTRACT

The study was undertaken on ten healthy buffalo calves. The ventral buccal gland was located opposite to lower cheek teeth on the lateral aspect of the mandible. The gland was enclosed in a fibrous capsule and was lobulated. It was further sub-divided into superior and inferior divisions. The parenchyma of both the divisions consisted of secretory units (adenomeres) and duct system. The secretory units of superior division included mucous, serous and mixed type of alveoli with serous demilunes capping the mucous end-pieces, while those of inferior division were purely serous in nature. The mucous cells contained a small amount of glycogen along with acidic and metachromatically reactive mucopolysaccharides, Sudanophilic lipids, acid phosphatase and alkaline phosphatase enzymes while serous cells were negative to all these histochemical reactions.

Key words: Histomorphology, histochemistry, adenomeres, buccal gland, buffalo

The saliva plays an important role in all domestic animals in general and ruminants in particular. In ruminants it facilitates mastication, deglutition and provides fluid environment for microbial digestion of ingesta as fore-stomach in these animals is devoid of secretory glands. The saliva for digestion is secreted from major and minor salivary glands, where the ventral buccal gland forms a part of the latter. The studies conducted on ventral buccal salivary gland are very scanty particularly in buffalo. The present investigation provides its detailed histoarchitecture and histochemistry.

MATERIALS AND METHODS

The study was carried out on ten healthy buffalo calves (1-1½ years old) of either sex. The tissues of ventral buccal glands from different regions were collected and processed for paraffin and frozen sectioning techniques as described earlier for dorsal buccal gland in buffalo (Gupta *et al.*, 2000). The sections were stained with the Harris' haematoxylin and eosin stain for routine histological studies, Weigert's method for elastic fibers, Gomori's silver stain for reticular

fibers, Crossman's trichrome stain for connective tissue and muscular fibers, methenamine silver argentaffin stain for argentaffin cells, periodic acid Schiffs stain with and without saliva treatment for mucopolysaccharides, Best's carmine stain for glycogen, alcian blue with metanil yellow for acid mucopolysaccharides, Mayer's mucicarmine stain for mucin, Sudan-black-B method for fat, Nile blue sulphate for acidic and neutral lipids, azo-dye method for acid phosphatase and azo-dye coupling technique for alkaline phosphatase. Micrometry was done with the help of linear calibrated ocular micrometer. The relative proportions of various components were recorded with the help of a net square ocular micrometer to calculate their percentage.

RESULTS AND DISCUSSION

The ventral buccal gland was compact and lobulated. It extended from angle of mouth to the rostral border of masseter muscle opposite lower cheek teeth on the lateral aspect of mandible in buffalo as reported in domestic animals (Habel, 1975, Parida and Das, 1991a). The ventral buccal gland was compound tubulo-alveolar enclosed in a fibrous capsule of varying

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thickness (Fig. 1) ranging from 69.93 to 113.22 μm with an average of $98.35 \pm 2.81 \mu\text{m}$. The septa given off from the capsule divided the parenchyma of the gland into lobes and lobules. Both the capsule and septa consisted of mainly collagen fibers with few reticular and scattered elastic fibers. A small amount of collagen and reticular fibers forming the interstitial tissue were dispersed in between the glandular end-pieces of the parenchyma. This architecture resembled to that of dorsal buccal gland in buffalo (Gupta *et al.*, 2000). The smooth muscle fibers reported in the capsule and stroma of major salivary glands of buffalo (Barnwal, 1978) and elastic fibers in the interstitial tissue of minor salivary glands in domestic ruminants (Parida and Das, 1991 b) could not be demonstrated in the present study. The ventral buccal gland exhibited distinct superior and inferior divisions.

The superior division of ventral buccal gland was comparatively small consisting of secretory units (adenomeres) and their duct system. The secretory units were comprised of mainly mucous (63.47%) and mixed (26.95%) with a few serous (9.58%) alveoli (Fig. 2). Few serous cells intermingled with mucous cells in the mixed alveoli and serous demilunes capping the mucous alveoli were also seen. These findings were similar to those for dorsal and middle buccal glands in buffalo (Gupta *et al.*, 2000, 2004). Parida and Das (1991 a) observed that the buccal glands in domestic ruminants were mixed type with mucous preponderance whereas, Trautmann and Fiebiger (1957) reported that the entire ventral buccal gland of cattle and its ventral portion in sheep and goat was purely serous.

The mucous alveoli were round, oval or irregular in shape measuring on an average 33.30 mm in diameter. The pyramidal type of cells lining the alveoli was supported by a PAS-positive basement membrane made up of reticular fibers. A few stellate shaped myoepithelial or basket cells were juxtaposed beneath the basement membrane. Parida and Das (1991 b) and Gupta *et al.* (2000) also reported that each acinus/alveolus of minor salivary gland in domestic ruminants was bound in a PAS-positive lamina composed of reticular fibers and myoepithelial cells. The pyramidal epithelial cells measured

13.32 mm to 23.31 mm with an average of $17.06 \pm 0.84 \text{ mm}$ in height. These cells were filled with slightly basophilic vacuolated cytoplasm and contained granular mucinocarminophilic material giving foamy appearance (Fig. 2 and 3). Kay (1960) in sheep and cattle and Gupta *et al.* (2000) in buffalo also mentioned the presence of mucicarmine positive granules in the mucous alveolar cells of buccal glands. This mucicarmine positive reaction may be due to the presence of acid prosthetic groups (highly complex sulphuric acid) in mucin (Clara, 1940, Gupta *et al.*, 2000, 2004). The nucleus was hyperchromatic and flat pushed towards the basement membrane. Occasionally argentaffin like cells having silver positive granules were also seen near the basement membrane like that in dorsal buccal gland in buffalo (Gupta *et al.*, 2000). These cells may be endocrine cells associated with the secretion of local hormones like serotonin, glucagon, catecholamines, secretin, pancreozymin and gastrin etc. (Philipson, 1982).

Histochemically the mucous cells contained strongly reactive PAS – positive coarse granular material (Fig. 4) which was slightly saliva labile and weakly reactive with Best's carmine stain indicating the presence of small amount of glycogen along with other mucopolysaccharides conforming with the earlier reports of Pearse (1968) and Gupta *et al.* (2000, 2004). The other mucopolysaccharides included metachromatically reactive and acid mucopolysaccharides as exhibited by strong reaction with mucicarmine (Fig. 3) and alcian blue (Fig. 5) stains respectively. A Sudanophilic non-specific fatty material was distributed throughout the cytoplasm whereas, Nile blue sulphate method failed to demonstrate the presence of acidic and neutral lipids in them. The acid phosphatase activity was strong and granular throughout the cell whereas mild to moderate alkaline phosphatase activity was limited to the basal region only.

The serous alveoli were scattered individually and formed 9.58 per cent of the secretory units. The alveoli were almost round and measured 21.64 mm to 31.63 mm in diameter with an average of $22.82 \pm 1.58 \text{ mm}$. Wedge or pear shaped cells lining the serous alveoli were supported by a basement membrane made up of

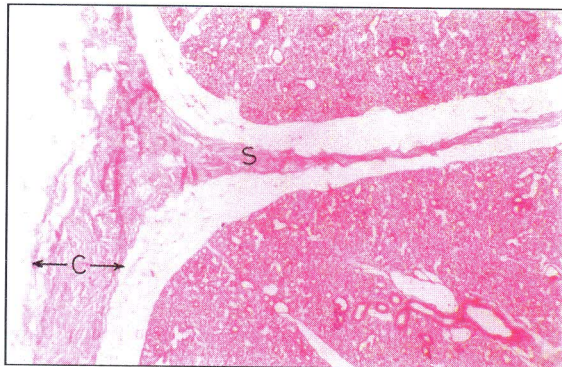


Fig.1. Ventral buccal gland showing capsule (C) and interlobar septa (S). (H. & E. x 20.)



Fig.4. Superior division of ventral buccal gland showing PAS - positive material in mucous alveoli (M). PAS-negative serous alveoli (S) can also be seen. (Periodic acid Schiff's method x 100.)

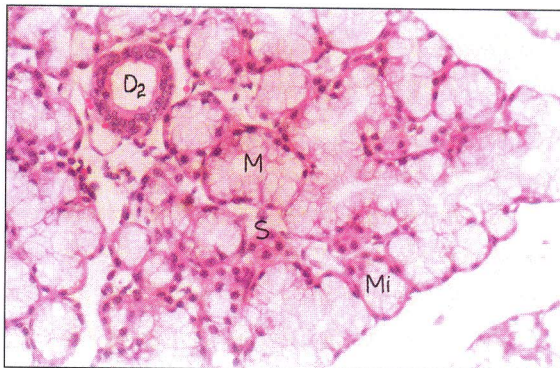


Fig.2. Superior division of ventral buccal gland showing mucous (M) serous (S), mixed (Mi) alveoli and intralobular duct (D2). (H. & E. x 100)

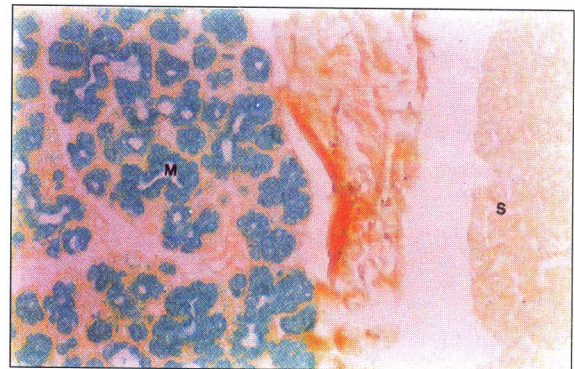


Fig.5. Ventral buccal gland showing Alcian blue positive material only in mucous alveoli of superior division and absent in the inferior division which is purely serous. (Alcian blue with metanil yellow stain x 50)



Fig.3. Superior division of ventral buccal gland showing mucicarmineophilic material in mucous alveoli (M) which is absent in serous alveoli (S) and ducts (D). (Mayer's mucicarmine stain x 100)

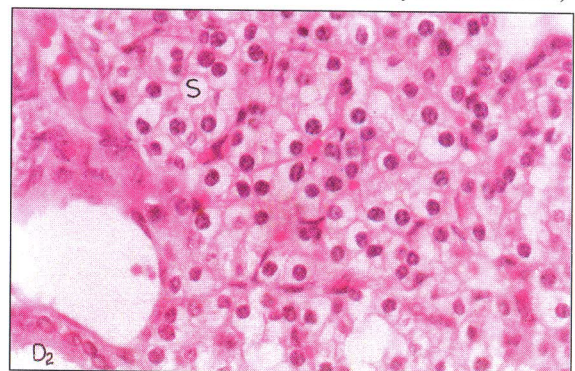


Fig.6. Inferior division of ventral buccal gland showing serous alveoli (S) lined with wedge of pear shaped cells and intralobular duct (D2). (H. & E. x 200)

reticular fibers and stellate shaped myoepithelial cells. The myoepithelial cells were said to be contractile which squeeze the lumen of alveoli for extrusion of secretory products (Parida, 1990, Stinson and Calhoun, 1993). The serous epithelial cells measured 9.25 ± 0.62 mm in height with a range of 6.66 mm to 9.99 mm.

The nuclei were round and vesicular type, which were located either in the centre or in the basal part of the cell depending upon the relative amount of secretion (Fig. 6). The cytoplasm was finely granular and lightly acidophilic in nature. These studies were in consonance with those described earlier (Gupta *et al.*, 2000, 2004).

Histochemically the serous cells were negative to all staining methods undertaken during the present study (Figs.3, 4 and 5), confirming earlier observations regarding absence of the histochemical constituents in the serous alveoli of buccal glands of ruminants and buffalo (Parida and Das, 1992, Gupta *et al.*, 2000, 2004).

Mixed alveoli and serous demilunes collectively constituted about 26.95 per cent of the secretory units. The mucous and serous cells intermingled with each other (Fig. 2). The cells absolutely resembled the corresponding cells in histomorphology and histochemistry described for mucous and serous alveoli respectively during the present investigation.

The inferior division of ventral buccal gland was comparatively larger in size as compared to the dorsal division of the gland and purely serous in nature (Fig. 6). Parida and Das (1991 a) in domestic animals and Trautmann and Fiebiger (1957) in sheep and goat also reported that the inferior division of ventral buccal gland was purely serous. The connective tissue septa consisting of collagenous and reticular fibers divided the parenchyma into lobes and lobules. Very fine fibers from septa extended in between the alveoli forming interstitial tissue. The serous alveoli were mostly round and resembled in histomorphology and histochemistry (Fig. 5) to the corresponding serous alveoli described for the superior division of the same gland.

REFERENCES

- Barnwal, A.K. (1978). Histological and histochemical studies on the salivary glands of Indian buffalo (*Bubalus bubalis*). Ph.D. thesis, Magadh University, Bodh Gaya.
- Clara, M. (1940). Untersuchungen Ober den farberischen Nachweis des Schleimes in den Drusenzellen des Menschen. Jahrb. Morph. U. mikrosk. Anat. Abt. II. Zeitschr. Mikrosk- anat.. Forsch **47**: 183-426. Cited in *Biol. Abst.* **16**: 1219.
- Gupta, M.K., Gupta, A.N. and Jain, R.K. (2000). Histomorphology and histochemistry of adenomeres of dorsal buccal gland in buffalo (*Bubalus bubalis*). *Indian J. Vet. Anat.* **12**: 162-168.
- Gupta, M.K., Gupta, A.N. and Jain, R.K. (2004). Histomorphology and histochemistry of adenomeres of middle buccal gland in buffalo (*Bubalus bubalis*). *Indian J. Anim. Sci.* **74**: 635-36.
- Habel, R.E. (1975). Ruminant digestive system. In : The Anatomy of the Domestic Animals. Getty, R (ed.) 5th edn. Vol.I, pp. 861-874. W.B. Saunders Co., Philadelphia, U.S.A.
- Kay, R.N.B. (1960). The rate of flow and composition of various salivary secretions in sheep and calves. *J. Physiol.* **150**: 515-537.
- Parida, S. (1990). Gross and histomorphological study of the minor salivary glands of domestic ruminants. M.V.Sc. thesis, Orissa University of Agriculture and Technology, Bhubaneswar.
- Parida, S. and Das, L.N. (1991 a). Gross and histological distribution of minor salivary glands in domestic ruminants. *Orissa Vet. J.* **16**: 109-117.
- Parida, S. and Das, L.N. (1991 b). The minor salivary glands of domestic ruminants. *J. Bombay Vet. Coll.* **3**: 47-52.
- Parida, S. and Das, L.N. (1992). Histochemical characterization of minor salivary glands of domestic ruminants. *Indian J. Vet. Anat.* **4**: 1-8.
- Pearse, A.G.E. (1968). Histochemistry, Theoretical and Applied, 3rd edn. Vol.I, pp. 495-575 and 710-736. J. & A. Churchill Ltd., London.
- Philipson, A.T. (1982). Ruminant digestion. In: Duke's Physiology of Domestic Animals. M.J. Swenson (ed.) 9th edn. Comstock Publishing Associates, London.
- Stinson, A.W. and Calhoun, M.L. (1993). Digestive system. In : Text Book of Veterinary Histology. H.D. Dellmann and E.M. Brown (eds.) 4th edn. pp. 161-163, Lea and Febiger, Philadelphia.
- Trautmann, A. and Fiebiger, J. (1957). Fundamentals of the Histology of Domestic Animals. Comstock Publishing Associates, Ithaca, New York.