

## HISTOMORPHOLOGICAL, HISTOCHEMICAL AND MICROMETRICAL STUDIES ON THE MIDDLE BUCCAL GLAND OF SHEEP (OVIS ARIES)

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### ABSTRACT

The tissues from the middle buccal gland of ten healthy adult sheep of either sex of local mixed breed were collected from rostral, middle and caudal regions and processed for paraffin and frozen sectioning techniques. The gland was compound tubuloalveolar comprising of secretory units (adenomeres) and their duct system. The adenomeres consisted of mainly mucous with few serous and some mixed type of alveoli. The alveolar and luminal diameters of secretory units were measured. The epithelial height was also more than that of dorsal and ventral buccal glands. The histochemistry revealed that the mucous cells contained mucosubstances, glycogen, mucopolysaccharides, mucin and sudanophilic lipids while serous cells were negative to all these histochemical reactions.

**Key words:** Histomorphology, histochemistry, micrometry, middle buccal gland, sheep

The major and minor salivary glands help in digestion of food through their saliva and provide an environment for microbial digestion of ingesta as forestomach in ruminants is devoid of secretory glands. A very little work has been conducted on minor salivary glands. Keeping in view the paucity of literature, the present work on histomorphology, histochemistry and micrometry was, therefore planned.

### MATERIALS AND METHODS

The present study was undertaken on ten healthy adult sheep of either sex of local mixed breed. The tissues of middle buccal gland from different regions were collected and processed for paraffin sectioning. The sections were stained with Harris' haematoxylin and eosin stain for histomorphological studies (Luna, 1968), Crossman's trichrome stain for collagen fibres (Crossman, 1937), Gomori's stain for reticular fibres, Weigert's method for elastic fibres, Alcian blue for mucosubstances (pH 2.5), PAS-Alcian blue method for mucosubstances (pH 2.5) Best's carmine method for glycogen McManus' PAS method for glycogen, Diastase digestion method, Mayer's mucicarmine method for mucin, Sudan black B method and Oil-red-O in propylene glycol method for fats (Luna, 1968), colloidal iron stain for acid mucopolysaccharides (Thomson and Hunt, 1966) and Nile blue method for neutral and acidic lipids

(Drury and Wallington, 1967). Micrometry was done with the help of an ocular micrometer.

### RESULTS AND DISCUSSION

The middle buccal gland was compound tubuloalveolar type gland as reported earlier by Stinson and Calhoun (1993), Banks (1993) and Gupta *et al.* (2000, 2002, 2004). It was encapsulated by a thick dense capsule having collagen, reticular and elastic fibres along with striated muscles in addition to fine blood capillaries and nerve fibres as described in domestic animals (Parida and Das, 1991) and buffalo (Gupta *et al.*, 2000, 2002, 2004). From the capsule the trabeculae or interlobular septae were emerging which divided the parenchyma into lobes and lobules. The majority of alveoli were purely mucous in nature and only a few serous or mixed types of alveoli were observed (Fig. 1).

The mucous alveoli were mainly round to oval shaped measuring 59.92 to 78.64  $\mu$  with an average of  $70.13 \pm 3.91 \mu$  in diameter. These were lined by pyramidal shaped cells with height varying from 13.30 to 21.50  $\mu$  (average  $18.21 \pm 1.16 \mu$ ). The nuclei were elongated, darkly basophilic and pushed towards the basement membrane. The alveolar lumen was wide with 6.70 to 16.24  $\mu$  in diameter (average  $10.98 \pm 0.81 \mu$ ). The chromatin material was very dense. The cytoplasm was finely granular, eosinophilic and presented vacuolated appearance because of washing of mucin. The mucous

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alveoli were surrounded by fine collagen, reticular and isolated elastic fibres, myoepithelial cells, fibroblasts and a few fine blood capillaries.

The serous alveoli were only few which were scattered in between the mucous alveoli. The alveolar diameter ranged from 19.22 to 29.80  $\mu$  (average  $24.31 \pm 1.98 \mu$ ). These were also lined by pyramidal shaped cells whose height varied from 6.84 to 17.75  $\mu$  (average  $10.91 \pm 1.13 \mu$ ). The nuclei were round to oval shaped mainly placed towards the lower basal portion of cell. The nuclei were comparatively less basophilic than those of the mucous alveoli. The cytoplasm of cell was more eosinophilic and finely granular. The lumen was narrow and the luminal diameter measured from 1.50 to 4.55  $\mu$  (average  $2.86 \pm 0.37 \mu$ ). The apices of these cells towards lumen contained large number of eosinophilic granules. These cells were also surrounded by myoepithelial cells, isolated collagen and reticular fibres. A few isolated alveoli possessed cells having characteristics of both mucous and serous cells.

Collagen fibres were distributed in the trabeculae or septae, alveoli and blood vessels, interlobular ducts, and between the fasciculi of the striated muscles. Large number of collagen fibres separated the lobes of the glands (Fig. 2). Elastic fibers were mainly present in tunica intima of blood vessels and fine reticular fibres were seen in the basement membrane. Histochemically, the mucous alveoli were strongly PAS positive which revealed the presence of acidic, neutral mucopolysaccharides, acetyl-galactosamine and glucuronic acid. The diastase treatment showed decrease

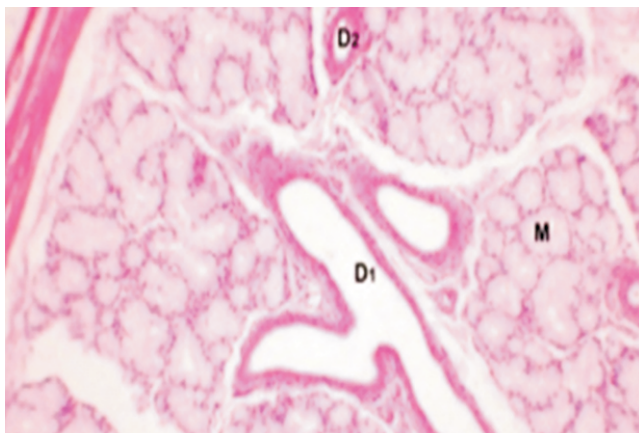


Fig 1. Photomicrograph of middle buccal gland showing the presence of mucous alveoli (M) in the glandular parenchyma. A large interlobular duct (D1) and a small intralobular duct (D2). (H. & E. x 100)

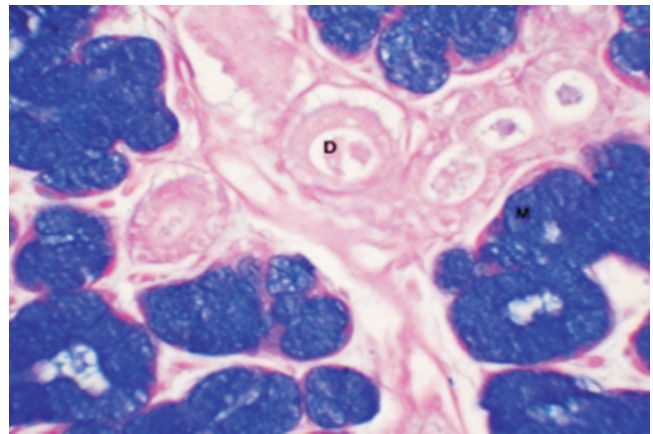


Fig 2. Photomicrograph of middle buccal gland showing strong PAS-AB positive reaction in mucous alveoli (M) whereas duct (D) showed negative reaction. (PAS-Alcian blue x 400)

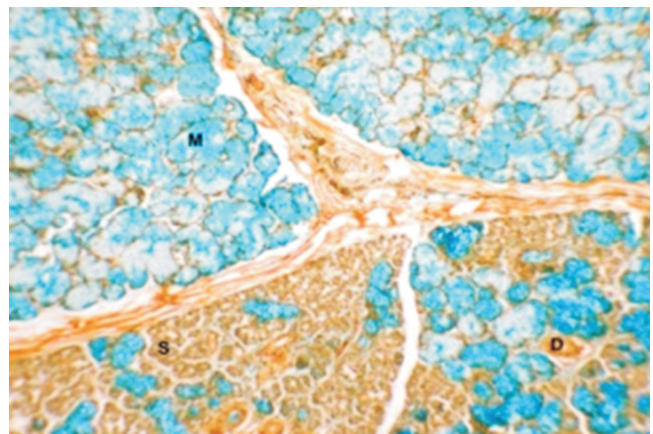


Fig 3. Photomicrograph of middle buccal gland showing the presence of colloidal iron positive material in mucous alveoli (M), but absent in serous alveoli (S) and small intralobular duct (D). (Colloidal iron method x 100)

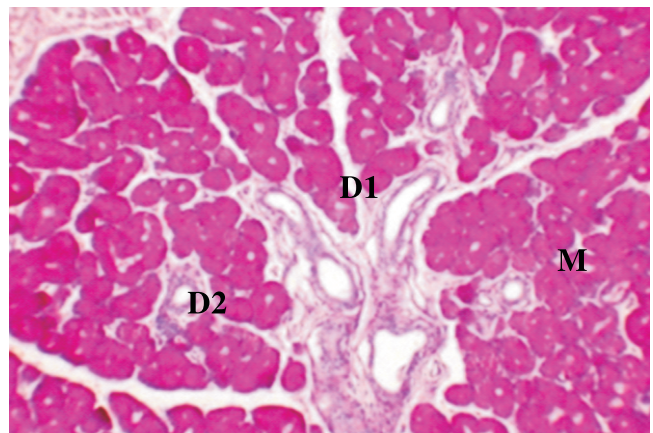


Fig 4. Photomicrograph of middle buccal gland showing strong PAS positive material in mucous alveoli (M) which was absent in both interlobular (D1) and intralobular (D2) ducts. (McManus' method x 100)

in PAS positivity indicating the presence of glycogen other than mucopolysaccharides. The mucous alveoli were strongly positive for acidic and neutral mucopolysaccharides by PAS-AB method having almost of equal distribution (Fig. 3). The mucous alveoli were strongly positive for mucosubstances by Alcian blue method and all ducts were negative (-) by this method. These alveoli showed moderate reaction for mucin. The reaction with mucicarmine indicated the presence of acid prosthetic groups (highly complex sulphuric acid) in mucin (Clara, 1940). Mucous alveoli showed moderate reaction for acid mucopolysaccharides by Colloidal iron method (Fig. 3). The colloidal iron staining character of these cells got support from the work in case of domestic ruminants (Parida and Das, 1992). The fatty material was unevenly distributed in the cytoplasm and showed mild reaction with Sudan black-B. Nile blue sulphate and Oil-red-O showed weak reactions for lipids. The intralobular and interlobular ducts were mainly PAS negative (Fig. 4). The cytoplasm of serous alveoli of middle buccal gland in sheep did not contain any histochemically reactive substance studied during the present investigation which was in agreement to the findings in buffalo (Gupta *et al.*, 2000, 2002, 2004) and in domestic ruminants (Parida and Das, 1992).

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