

## Fine Structure of the Submucosal Layer of The Dog's Cervical Esophagus

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Received: 18 July 2025; Accepted: 15 September 2025

### ABSTRACT

Six samples of cervical oesophagus were collected from apparently healthy adult dogs that died in different road accidents. The samples were processed for a transmission electron microscopy study. The submucosal layer of the canine esophagus consists of compound, muco-serous glands, fibroblasts, macrophages, plasma cells, lymphocytes, and connective tissue fibers, including collagen, reticular, and elastic fibers. The mucous acini displayed mucous and subsidiary cells with large lucent granules and small dense granules, respectively. The serous acinar cells had a nucleus, electron-dense granules of varying sizes and appearances, a Golgi apparatus, and abundant endoplasmic reticulum. The myoepithelial cells were flat, spindle-shaped, rich in tonofilaments, and rested on the basement membrane of the serous or mucous acini. The cells lining the duct ranged from low cuboidal cells to two-layered forms, becoming stratified at the opening into the lumen of the esophagus. The intra-lobar duct showed cells with prominent centrally located nuclei, electron-dense secretory granules, a greater number of mitochondria, polyribosomes, and endoplasmic reticulum. The plasma membrane of the cuboidal cells located towards the lumen of the duct showed blebs. The cells of the intralobar duct were joined to one another by desmosomes and tight junctions.

**Keywords:** esophagus, submucosa, mucous acini, serous acini, myoepithelial cell

### INTRODUCTION

The esophageal tube actively transports the chewed food, mucus, and saliva to the stomach. The wall of the esophagus consists of tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa layers. The tunica submucosa is a thick layer of loose connective tissue, located between the tunica mucosa and tunica muscularis. The function of esophageal glands is to lubricate and to protect the underlying layer from the dry food eaten (Al-Shabebi *et al.*, 2019), and the secretion of the submucosal glands neutralizes the acid produced in the stomach. The presence or absence of the glands and the extent of distribution of the submucosal glands vary with the diet. The submucosal glands were all along the length of the oesophagus of the dog from the pharyngo-oesophageal region to the cardia region (Botlagunta and Kedari, 2023). In the pig oesophagus, the glandular layer began to fade out at about the distal thoracic esophagus (Kedari *et al.*, 2023a). The ultrastructure of oesophageal

submucosal glands in adult pigs was studied by Kedari *et al.* (2023b). The present study aimed to record the ultrastructural characteristics of the submucosa in dogs, considering the abundant variations among species and within the regions of the oesophagus.

### MATERIALS AND METHODS

Six samples of cervical oesophagus were collected from apparently healthy adult dogs that died in different road accidents. The present work was conducted at the Department of Veterinary Anatomy, College of Veterinary Science, Tirupati, and the National Institute of Mental Health and Neurosciences (NIMHANS), Electron Microscopy Lab, Bengaluru. The samples of 2 mm size from the cervical esophagus of the dog were fixed immediately in 3% glutaraldehyde in 0.1M phosphate buffer (pH 7.2) for 24 hours at 4 °C and post-fixed in 2% aqueous osmium tetroxide for 4 hours. Then, they were dehydrated in ascending grades of alcohol for 1 hour each at 4°C. Subsequently, the samples were transferred into a mixture of 95% alcohol and 2% uranyl acetate for one hour at 4°C. They were then fully dehydrated with two changes of absolute alcohol for 30 minutes

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each. The samples were transferred to propylene oxide with two changes of 15 minutes each at room temperature and infiltrated with a 1:1 mixture of 10 ml propylene oxide and 10 ml Araldite resin overnight at room temperature. Afterwards, they were infiltrated with a 1:2 mixture of 5 ml propylene oxide and 15 ml Araldite resin overnight at room temperature and embedded in pure Araldite resin for four and a half hours at room temperature. Semi-thin sections were cut using an ultramicrotome (Leica EMUC7). The toluidine blue-stained semi-thin sections were observed under a light microscope. Ultra-thin (80 nm) sections were obtained from the desired areas and mounted onto copper grids. These sections were stained with saturated uranyl acetate for twenty-five minutes and counter-stained with 4% lead citrate for five minutes. They were then examined under various magnifications using transmission electron microscopy (TEM-1400 Plus-Jeol).

## RESULTS AND DISCUSSION

The tunica submucosa consisted of loose connective tissue, situated between the lamina muscularis mucosae and tunica muscularis. The submucosal layer of the canine oesophagus included fibroblasts, macrophages, plasma cells, lymphocytes, glands, occasional autonomic ganglionic cells, blood vessels, nerves, adipose tissue, and connective tissue fibers such as collagen, reticular, and elastic fibers, as reported by Botlagunta and Kedari (2023).

The submucosal glands were typical, compound, tubuloalveolar, comprised predominantly of mucous alveoli, a few serous acini, and serous demilunes, similar to the observations of Dawood *et al.* (2022) in local Iraqi dogs. Botlagunta and Kedari (2023) reported that in dogs, the submucosa contained mucous glands throughout the length of the esophagus.

Mucous acinar cells were pyramidal, with a basal nucleus (Figs. 1 and 5). Numerous secretory vesicles of varying electron densities and sizes occupied the entire cell. In some granules, the central area was electron dense, surrounded by a lucent region. The empty granules appeared foamy. Subsidiary secretory cells were located between the mucous acini or at the junction of the mucous acini with the duct. The secretory granules differ from those of the mucous cells, being smaller, denser, and homogeneous (Fig.3). Al Yassin *et al.*, (1977) identified two types of subsidiary cells in the mucous

acini: one with smaller, denser granules, and a second with no granules, found in the submucosal glands of the human esophagus. Oncocytes were the specialized cells found between the duct and acini in the human esophageal submucosa with characteristic, packed round, elongated, or oval mitochondria, with their free surface showing microvilli, and the rest of the surface attached to other acinar cells through desmosomes (Geobes *et al.*, 1988).

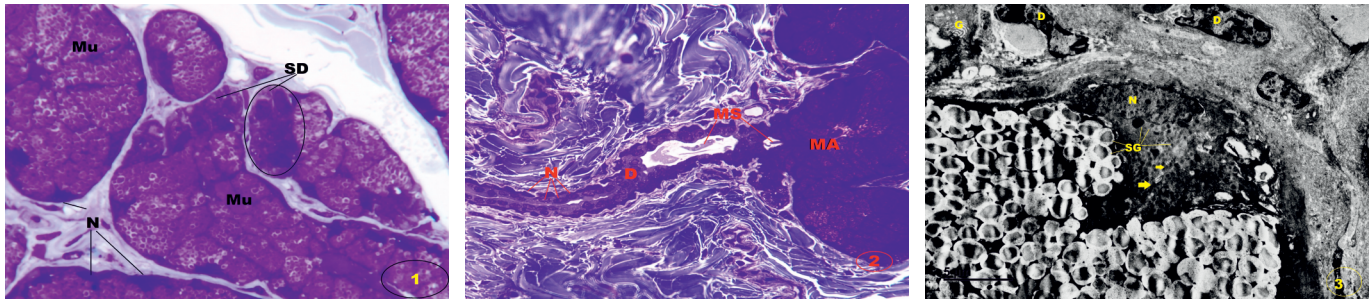
The serous acinar cells exhibited a nucleus, electron-dense granules of heterogeneous size and appearance, a Golgi apparatus, and abundant endoplasmic reticulum (Fig. 4), which was similar to the observations of Borthwick *et al.* (1999) in murine submucosal glands. The cells of serous acini are connected to adjacent cells by desmosomes. Some of the serous cells were positioned above the mucous acini as demilunes. The myoepithelial cells are flat, spindle-shaped, and rests on the basement membrane of serous or mucous acini, surrounding these acini. The cell cytoplasm contained mitochondria and dense bundles of tonofilaments (Figs. 4 and 5).

The cells lining the ducts range from low cuboidal cells (Figs. 3 and 6) through two-layered (Fig. 2), to a stratified layer at the opening into the lumen of the oesophagus. Similar observations were made in the submucosal glands of the pig by Kedari *et al.* (2023).

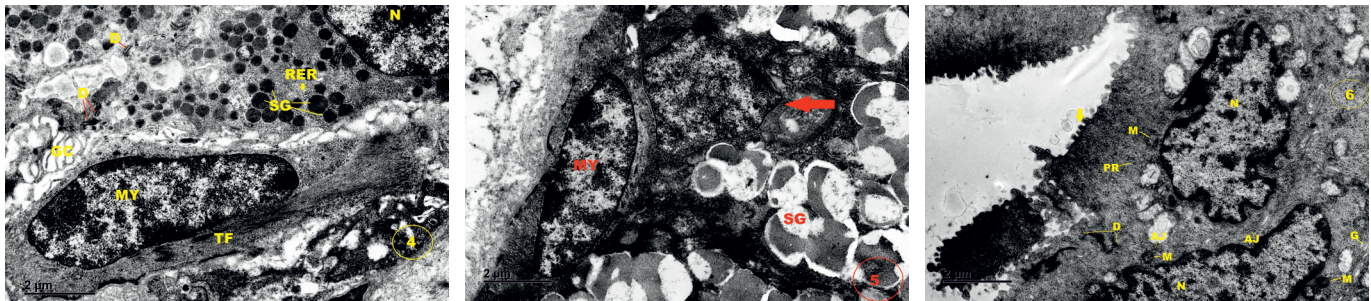
The connective tissue of the submucosa consisted of fenestrated capillaries, which showed the attenuated cell membranes of endothelial cells (Fig.7). According to Eurell and Frappier (2013), the circular fenestrae are 60-80 nm in diameter. The submucosal connective tissue had fibroblasts with large euchromatic nuclei, abundant rough endoplasmic reticulum, and mitochondria (Fig.8). An oval mast cell had a prominent, centrally located nucleus and membrane-bound, fine secretory granules (Fig.9). The plasma cell had an eccentrically placed nucleus with heterochromatin arranged peripherally, giving it a cartwheel-like appearance. The plasma cell exhibited abundant rough endoplasmic reticulum, a Golgi apparatus, spherical inclusions known as Russell bodies, and few mitochondria (Fig.10), consistent with the findings of Eurell and Frappier (2013).

## ACKNOWLEDGEMENT

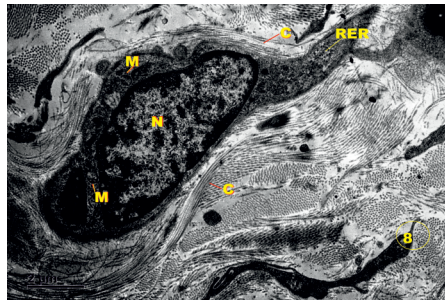
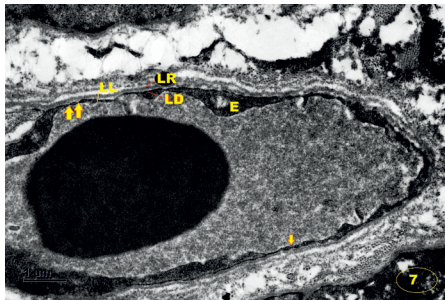
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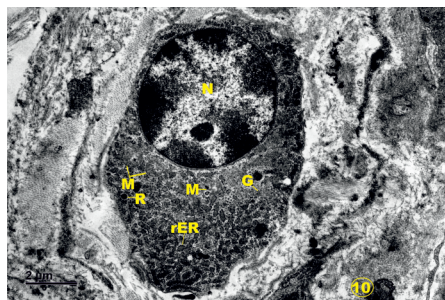
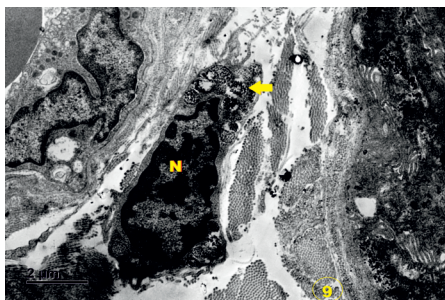
**Fig. 1:** Photomicrograph showing the Mucous acini (Mu) and serous demilunes (SD). N: nucleus. Toluidine blue-stained resin-embedded semithin section, 1000X; **Fig. 2:** Photomicrograph showing the Mucous acini (MA) and its secretion (MS) draining into the duct (D) lined with stratified cuboidal epithelium. N: nucleus. Toluidine blue-stained resin-embedded semithin section 1000X; **Fig. 3:** Electron micrograph showing the variation in the secretory granules (SG) of active mucous cells (lucent) and subsidiary secretory cells (smaller and denser). Both cells were connected by desmosomes (arrow). D- Nucleus of the ductal cuboidal epithelium 800X.



**Fig. 4:** Electron micrograph showing the serous cell with a prominent nucleus (N) and electron-dense secretory granules (SG) of various sizes in the submucosal gland of the oesophagus in a Dog. MY – myoepithelial cell, rER – Rough endoplasmic reticulum, GC – Golgi complex, TF-tonofilaments, D-desmosomes. 2000X; **Fig. 5:** Electron micrograph showing a mucous secretory cell with a basal location of nucleus (arrow) and a myoepithelial cell (MY) resting on the basement membrane of the acinar cell. SG-secretory granules 2000X; **Fig. 6:** Electron micrograph showing the cuboidal duct cells with a central nucleus(N), Blebs (Arrow) towards the luminal surface. M-mitochondria, PR-poly ribosomes, G-Golgi apparatus, AJ-adherent junctions, D-desmosomes 2000X.



**Fig. 7:** Electron micrograph showing the fenestrated capillary present in the submucosa of the Dog. E-endothelial cell, LL-lamina lucida, LR-Lamina reticularis, LD-lamina densa, arrows represent diaphragm. 2500X; **Fig. 8:** Electron micrograph showing the fibroblast with nucleus (N), rough endoplasmic reticulum (RER), mitochondria (M), and abundant collagen bundle (c) in the connective tissue of submucosa. 1500X;



**Fig. 9:** Electron micrograph showing the oval mast cell with heterochromatic nucleus(N) and fine granules(Arrow). 1500X; **Fig. 10:** Electron micrograph showing the plasma cell with cartwheel-like nucleus (N), rough endoplasmic reticulum (rER), mitochondria (M), G-Golgi apparatus, and R-Russel body.

Health and Neurosciences (NIMHANS) Electron Microscopy Lab for providing the facility for this study.

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