

Light and electron microscopic studies of pharynx and oesophagus of barbus sharpyi fish

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The histomorphological studies of Pharynx and oesophagus of barbus Sharpyi have been reported. The pharynx has two lateral pharyngeal bones each with 3 rows of pharyngeal teeth arranged in the dental formula: 20305-50302 in it's cranial part. The epithelium of pharynx was thick nonkeratinized stratified type and 144.6±21.2m thick. The mucous cells were seen scattered in this epithelium. The were deep branched crypts in epithelium that depth and the branching of crypts increased near the oesophagus. The bases of the crypts terminated into large mucous acini. Electron microscopic study showed that the mucous columnar cells of pharynx and oesophagus glands contained large amounts of large secretory granules. The basal cells also seen in these glands. The apical cytoplasm of basal cells containe mucous granules, which shows that the basal cell are able to change to mucous cells. The epithelium had taste buds. The muscularis mucosa was absent. Tunica muscularis

comprised of striated muscle cells. Oesophagus has two strong and thick sphincters ie, cranial or pharyngeo-oesophageal and caudal or bulbo-oesophageal. The mucosal epithelium was nonkeratinized stratified squamous type and had a few scattered goblet cells and a few taste buds in the cranial part. Numerous oesophageal mucous glands were seen in lamina properia-submucosa. Seromucous glands were seen in the terminal portion of oesophagus, near the opening of swim bladder. Tunica muscularis comprised of striated muscularis tissue, which was arranged in two layers, thin internal (longitudinal) and thick external (circular). In terminal portion, the scattered smooth muscle fibers were also seen. At the point connection of oesophagus with intestinal bulb, arrangement of cellular muscle fibers was reversed.

key words : Barbus sharpyi, Pharynx, Oesophagus, Histology

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Barbus sharpeyi is a species of cyprinidea family that is found in the rivers of Iran, Iraq, Suria and Turkey. Its native name in Iran is Beni and it is well adapted to the hard conditions of this area. It feeds particulary herbs. There is a big difference in the anatomical structure of various species of the Cyprinidea family. No information is available about the elementary canal of *Barbus Sharpeyi*, Hence, this project was undertaken to study the pharynx and oesophagus of this fish.

Material and Methods

Twelve live female fishes collected from " Hore of Shadegan " area of Khuzestan state of Iran about 1000Km South of Tehran, formed the material for this study. The fishes each one measured (31.5 ± 4.2 Cm). The pharynx and oesophagus of this fishes were collected by autopsy immediately and their pieces were fixed in 10% formal saline. Section of the tissues made by routine paraffine embedding method and stained by H&E and PAS staining technique for histological, morphological and histochemical studies. For electron microscopic studies, Pharynx and Oesophageal sections were made by TEM specimen prepration method.

Result

Pharynx

The pharynx is funnel shaped, with 1.27 ± 0.29 Cm long. In the cranial part, there are two lateral pharyngeal bones each with 3 rows of pharyngeal teeth. The caudal part is separated from the oesophagus by pharyngio-oesophageal sphincter.

Microscopically dental buds were seen in the pharyngeal wall these buds are devoid of enamel (Fig. 1). Histological structure of pharynx comprised of four tunics; Mucosa, Submucosa, Muscularis and Advantitia.

The mucosa comprised of epithelium and lamina properia. The mucularis mucosa was absent. The epithelium was nonkeratinized stratified type and 144.6 ± 21.2 μ m thick. The mucous cells were seen scattered in this epithelium. There were deep branched crypts in epithelium. The depth and branching of crypts increased towards the terminal portion of the pharynx. The bases of crypts terminating into large mucous acini (Fig. 2, 3).

The epithelium had taste buds, and their structure showed three types of cells ie. spindle shaped cells with large and round or ovaid euchromatinic nucleus, spindle shaped cells with smaller and elongated heterochromatinic nucleus and basal cells the spindle shaped cells have acidophilic cytoplasm with numerous dark granules (Fig. 4).

Lamina properia is loose connective tissue that had numerous simple mucous glands, it continued with submucosa (Fig. 2).

Tunica muscularis comprised of striated muscle fibers and had a thickness of 3170 ± 603.7 μ m which increased towards terminal portion. Although the layers of muscles were not comletly regular but the cells of internal layer mainly had a longitudinal arrangement with 2637 ± 778.5 μ m thick, and the external layer had mainly a circular arrangement with 478 ± 147.8 μ m thick (Fig. 5).



Fig. 1. Photomicrograph of Pharynx, Showing a pharyngeal dental bud, (H&E $\times 3.2$)



Fig. 2. Photomicrograph of Pharynx, Showing branched deep crypts that bases of its branched terminated into mucous acinar gland, (PAS, $\times 3.2$)



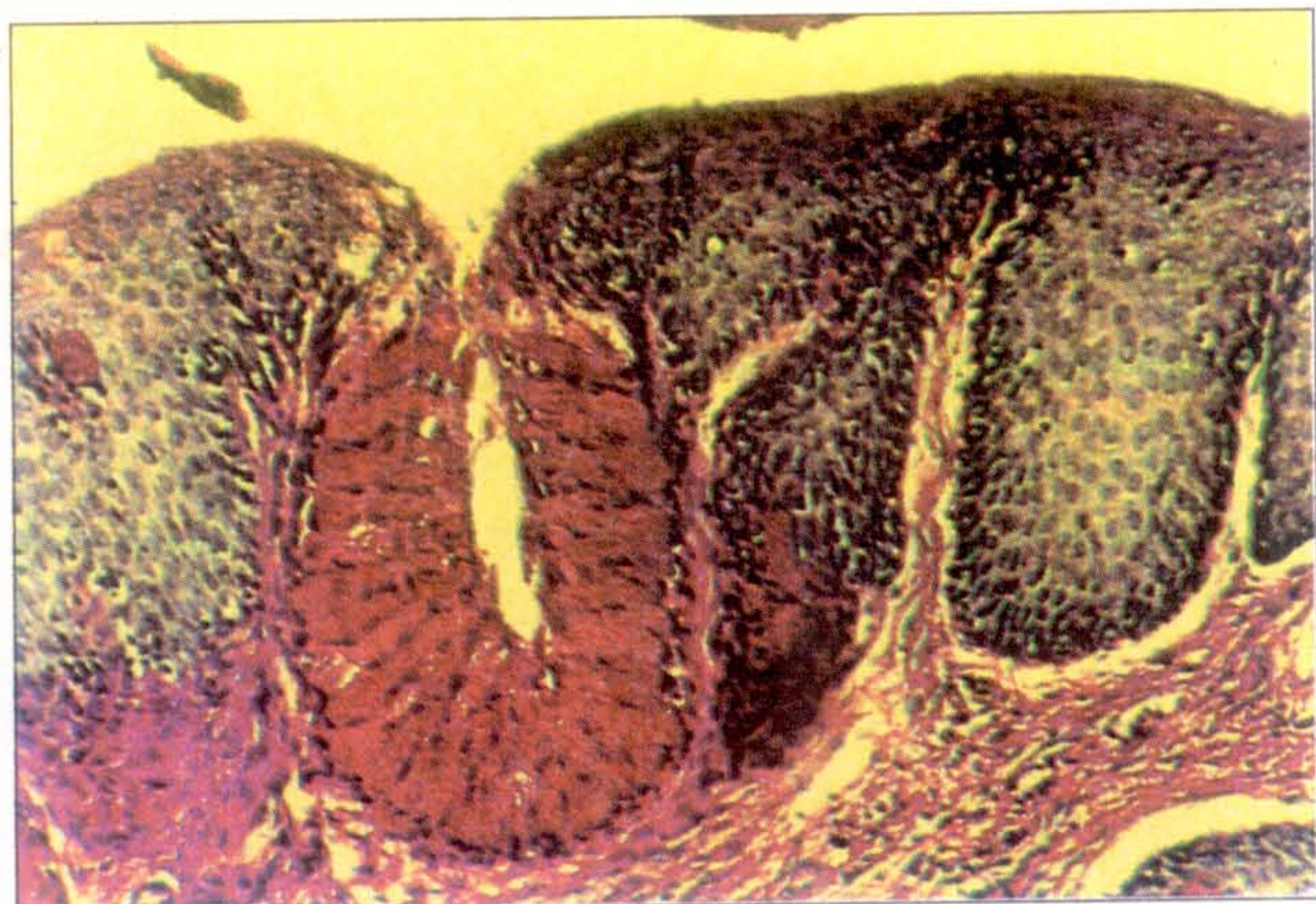


Fig. 3. Photomicrograph of pharynx, showing nonkeratinized squamous epithelium and a mucous acinar gland (PAS, x20)

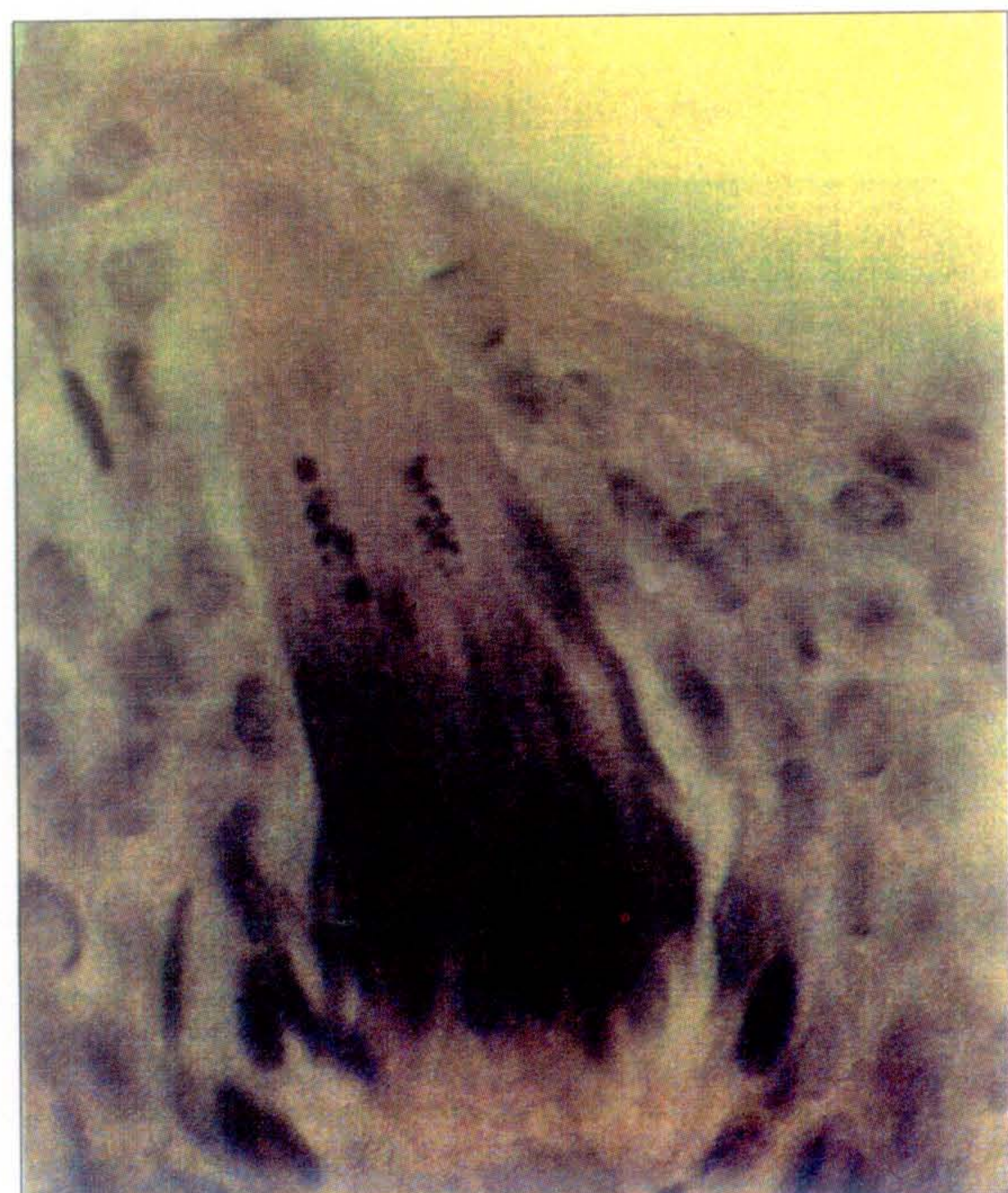


Fig. 4. Photomicrograph of pharynx, showing a test bud within lining epithelium. Which their cells contain large cytoplasmic granules (arrow), (H&E x3.2)

These layers were completely regular in the terminal portion of pharynx, near the oesophagus, where their thickness increased.

Oesophagus :

The oesophagus is short muscular tube (length 1.01 ± 0.9 Cm), that connected the pharynx to the intestinal bulb. Oesophagus has two strong and thick sphincter one in the cranial portion inside its connection with pharynx (length $792 \pm 141 \mu\text{m}$) (cranial or pharyngo-oesophageal sphincter) and the other in caudal portion inside its connection with intestinal bulb (length $1520.2 \pm 126 \mu\text{m}$), (Caudal or bulbo-oesophageal sphincter). The caudal sphincter is longer and stronger than the cranial sphincter (Fig. 6, 7). The cranial and caudal sphincters comprised mostly by circular muscular layer.



Fig. 5. Photomicrograph of pharynx, showing longitudinal (internal) circular (external) muscular layers (x3.2 H&E).

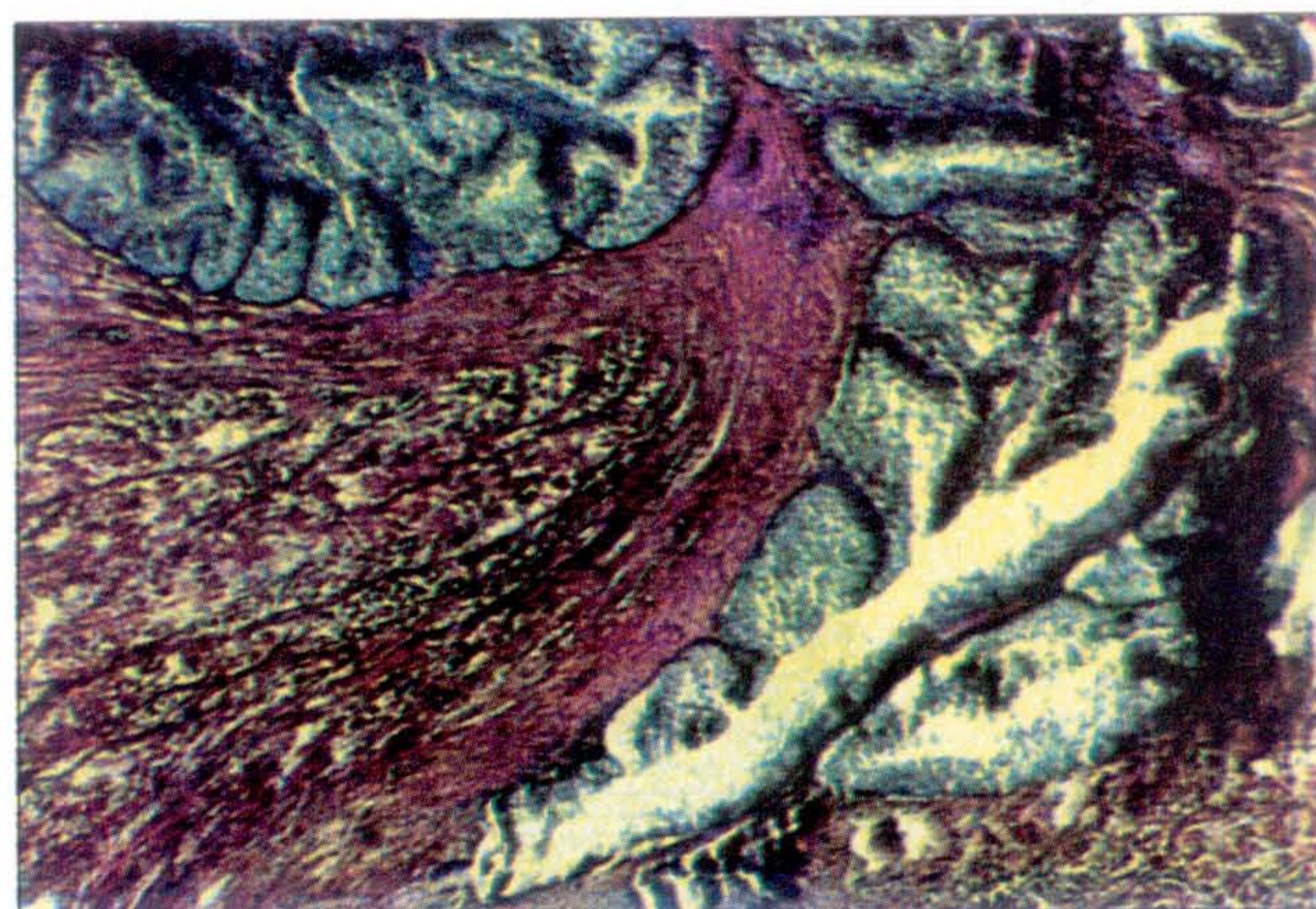


Fig. 6. Photomicrograph of the cranial or pharyngo-oesophageal sphincter (x3.2 H&E).

The mucosa has high longitudinal primary folds (length $839.4 \pm 80.5 \mu\text{m}$), which have smaller secondary folds (Fig. 8).

The duct of swim bladder opened in the terminal portion of oesophagus. Histological structure of oesophagus comprised of four tunics ie, mucosa, submucosa, muscularis and serosa. Mucosa comprised of epithelium and lamina propria. The muscularis mucosa was absent. The epithelium was nonkeratinized stratified squamous type and its thickness was $112 \pm 5.4 \mu\text{m}$ and a few scattered goblet cells (Fig. 9) and a few taste buds in cranial part, (more in the apical of primary mucosal folds), (Fig. 10). Lamina propria was a loose connective tissue continuous with submucosa because of absent of muscularis mucosa. Numerous oesophageal mucous glands were seen in lamina propria-submucosa. The





Fig. 7. Photomicrograph of the caudal or bulbo-oesophageal sphincter ($\times 3.2$ H&E).

glands arise from epithelium and are arranged in bundles. The glands were seen more in the lamina propria than in the submucosa (Fig. 8). Seromucous glands were seen in the terminal portion of oesophagus, near the opening of swim bladder (Fig. 11).

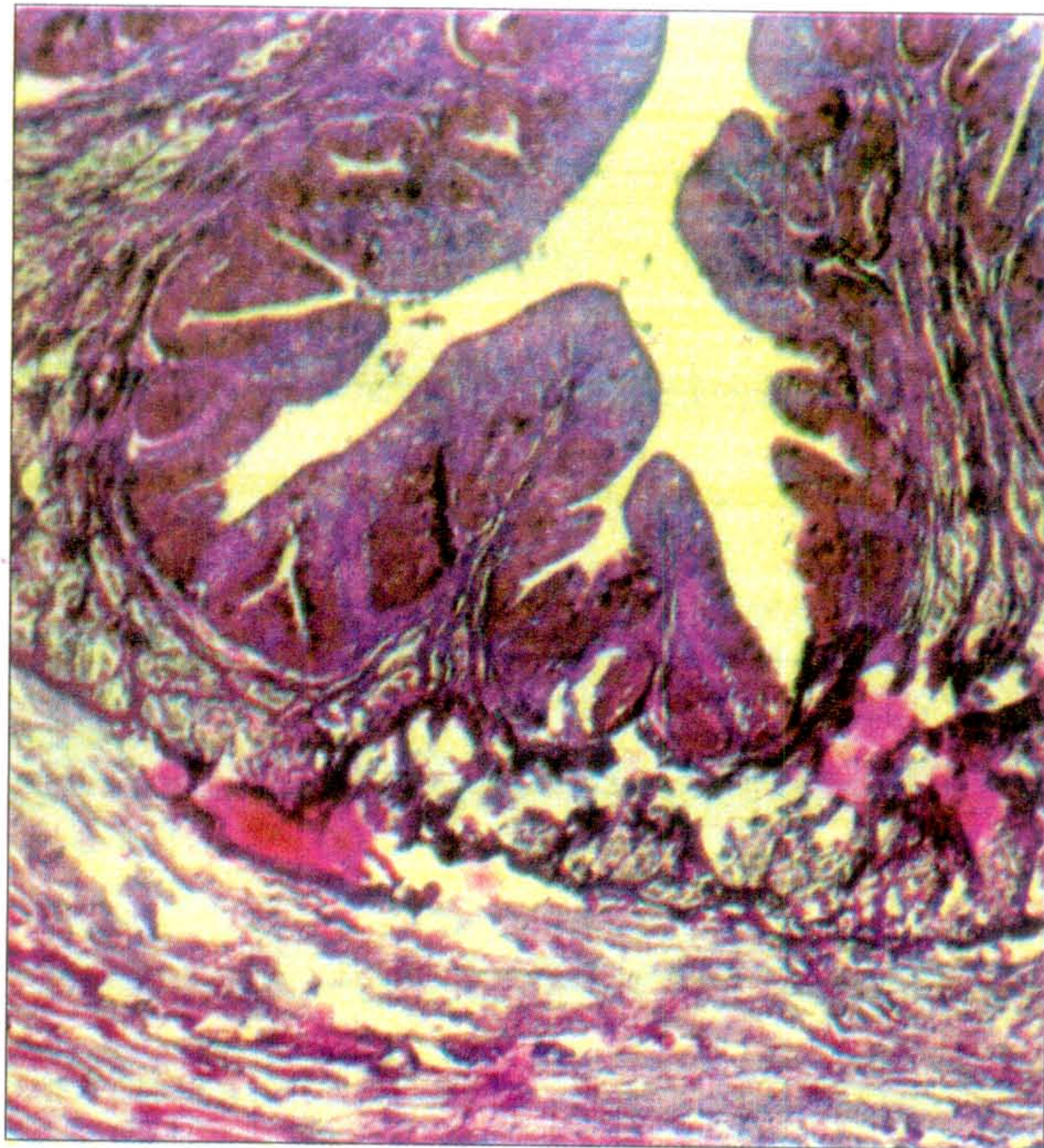


Fig. 8. Photomicrograph of oesophagus, showing the mucosal folds and mucous acinar glands ($\times 10$ PAS).

Tunica muscularis, comprised of striated muscular tissue which was arranged in two layers, thin internal (With longitudinal arrangement of muscle fibers), and thick external layer (with circular arrangement of muscle fibers), (Fig. 12). The thickness of internal and external layers was 396 ± 63.6 and $1441 \pm 220.5 \mu\text{m}$ respectively. The thickness of internal layer gradually decreased

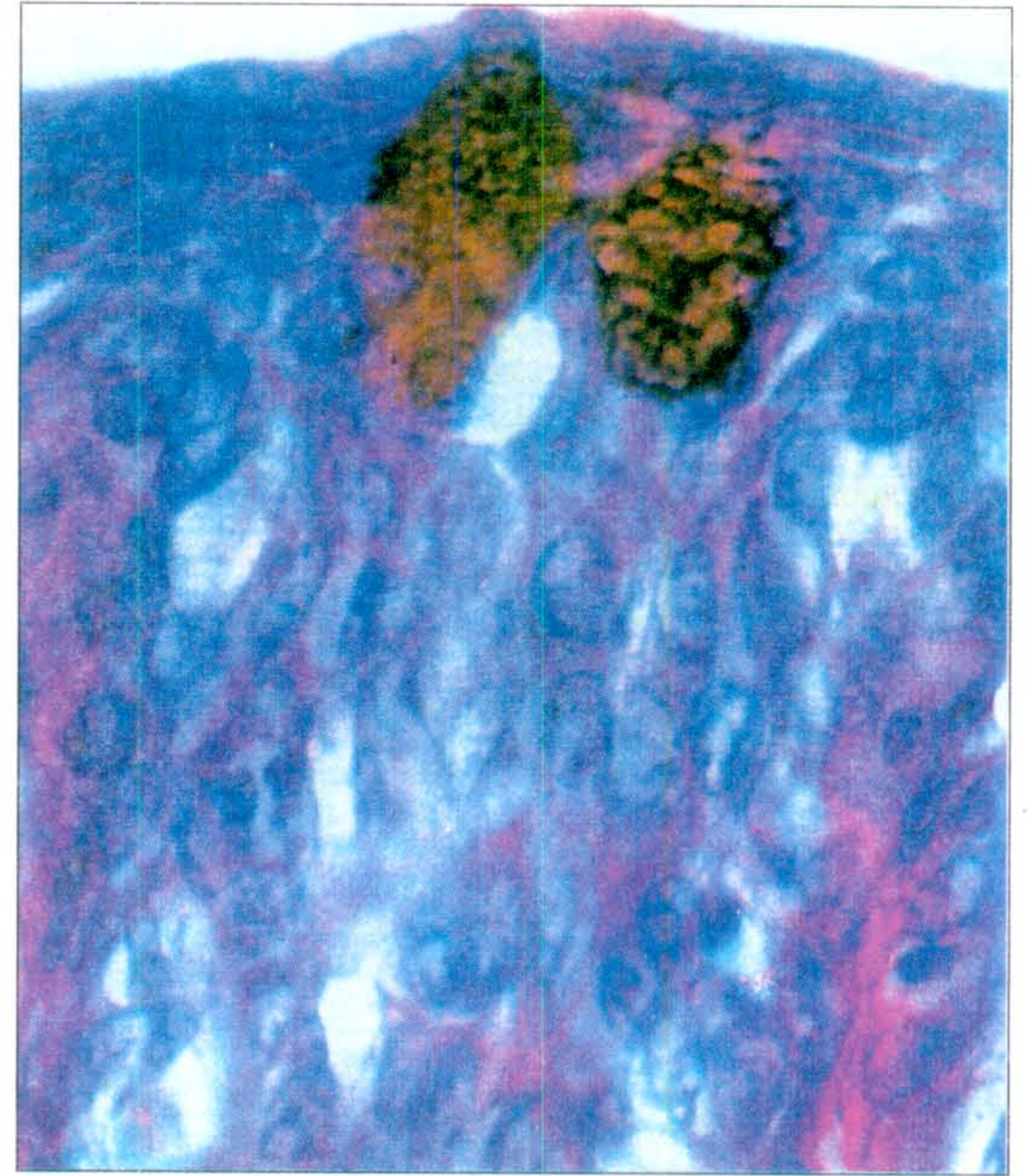


Fig. 9. Photomicrograph of oesophagus, showing the goblet cells, within the nonkeratinized stratified squamous



Fig. 10. Photomicrograph of oesophagus, showing a taste bud within the lining epithelium, which have three type of cells, ie, light cell (L) dark cell (D) and basal cell (B) ($\times 100$ PAS).

towards the terminal portion of oesophagus and finally discontinuous at the end. The terminal portion of oesophagus the scattered smooth muscle fibers were seen also. At the point connection of the oesophagus with the intestinal bulb, the internal layer of muscle fibers has a circular arrangement and the external layer of muscle fibers has a longitudinal arrangement.



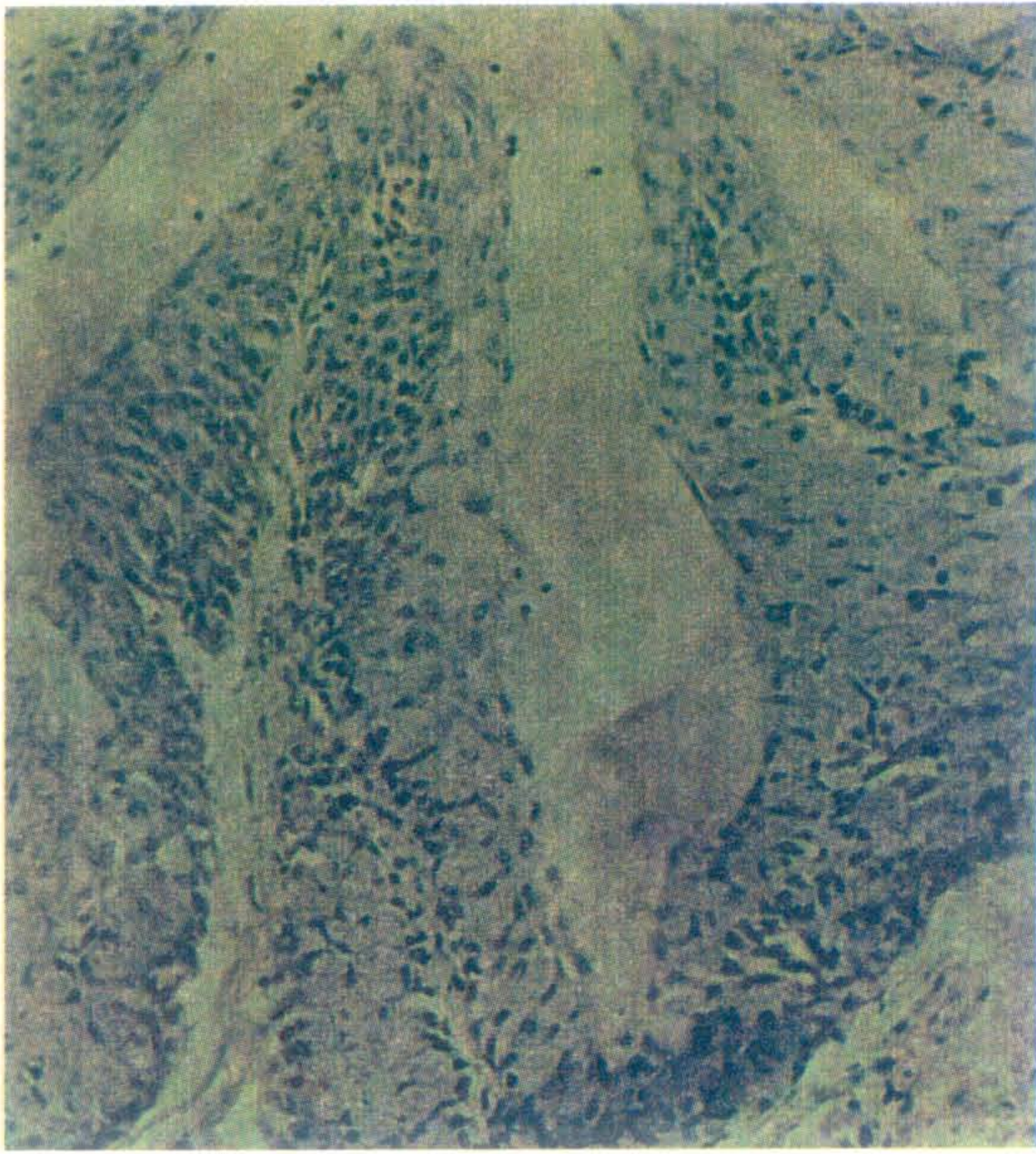


Fig. 11. Photomicrograph of terminal portion of the oesophagus, showing a seromucous gland within lamina propria ($\times 40$ H&E).

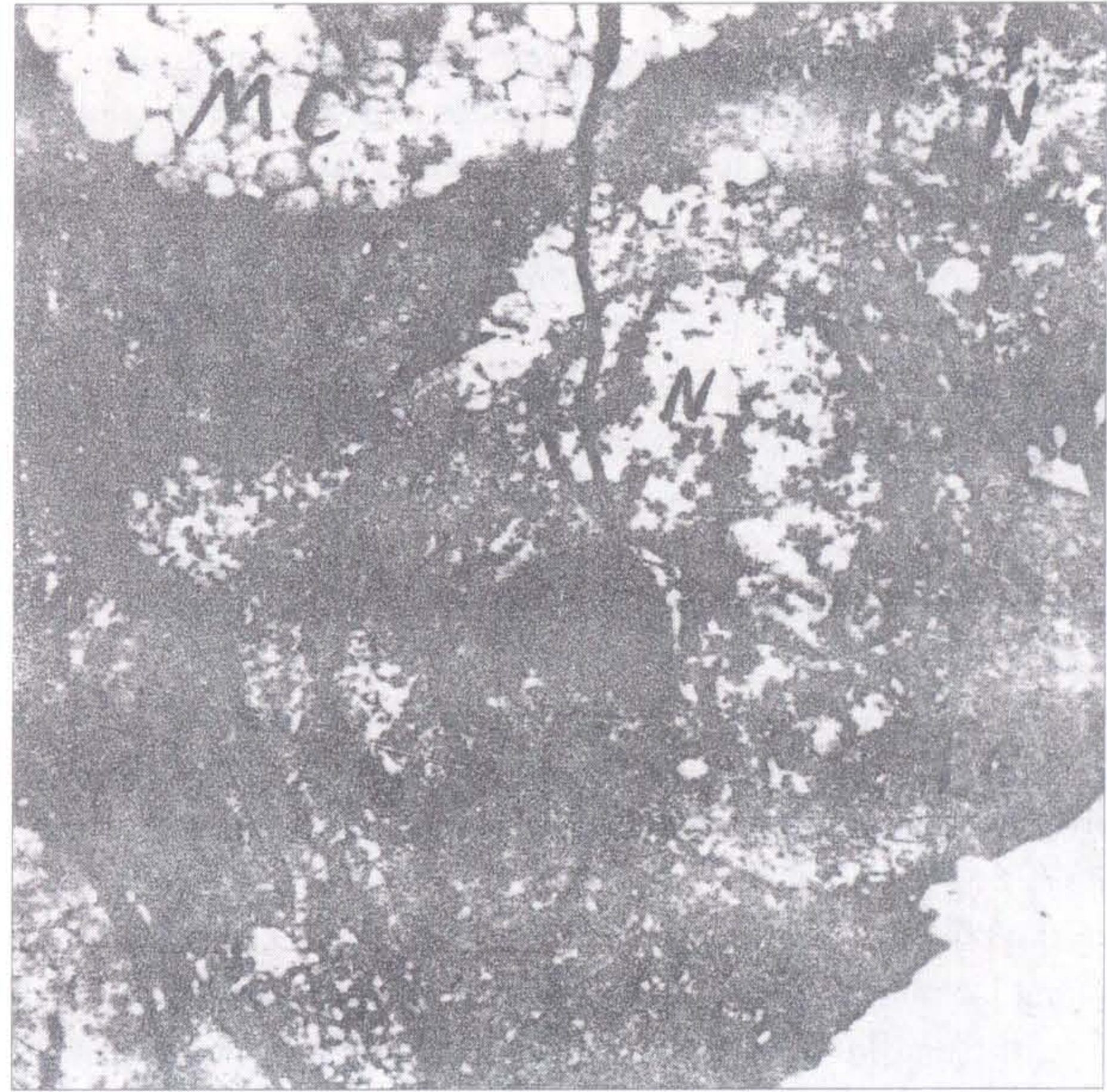


Fig. 13. Electron micrograph of oesophageal mucous cell, showing secretory granules (sg), $\times 3600$.

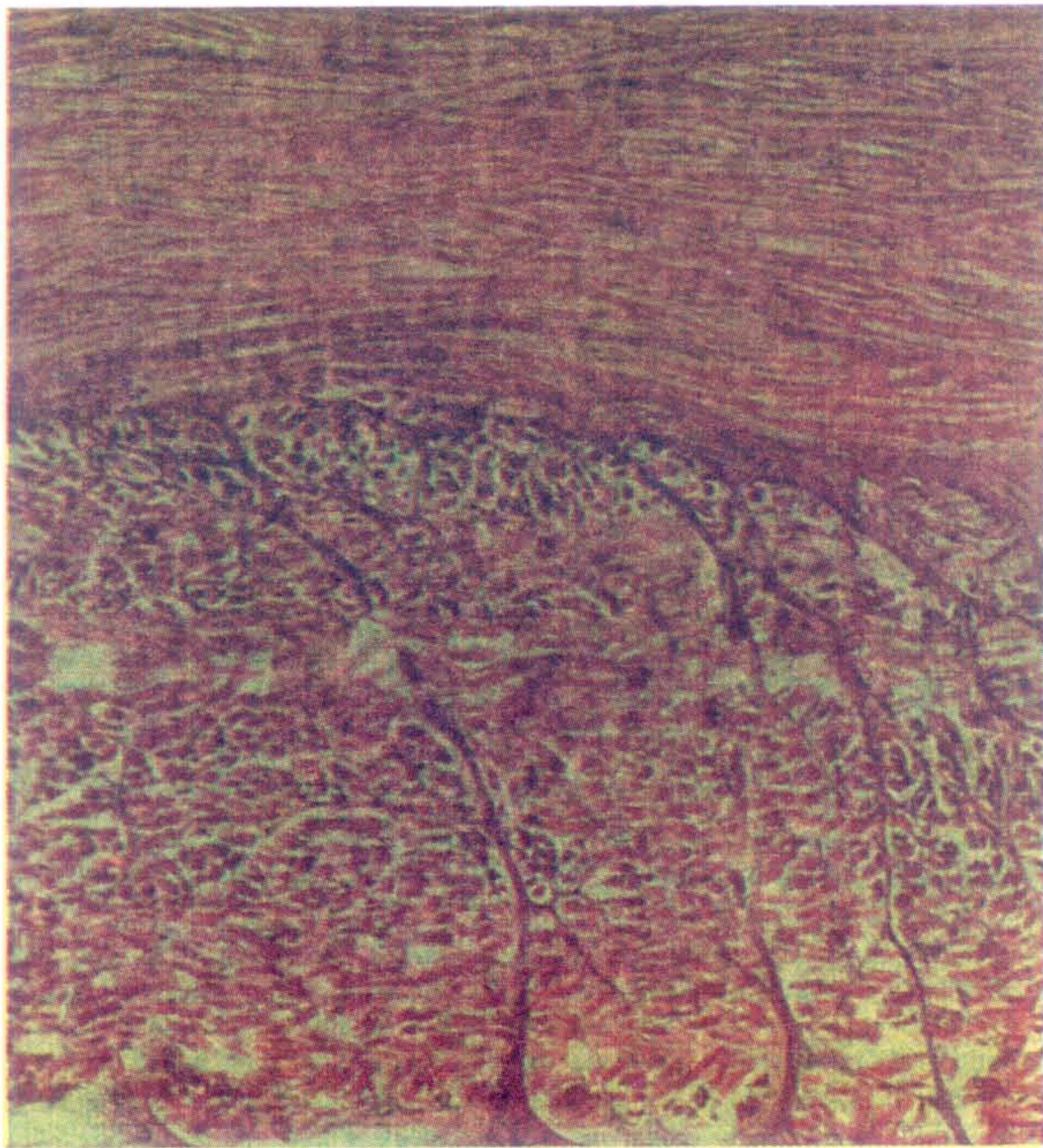


Fig. 12. Photomicrograph of the terminal portion of the oesophagus (longitudinal section), showing longitudinal (internal) and circular (external) muscle layers ($\times 3.2$ H&E).



Fig. 14. Electron micrograph of pharyngeal mucous acini, showing basal cell (BC) with euchromatin nucleus and apical mucous secretory granules mucous cell (MC), $\times 6000$.

Electron microscopic study showed that the mucous columnar cells of pharynx and oesophagus glands contained large secretory granules (Fig. 13). The basal cells also seen in these glands. The apical cytoplasm of basal cells contained mucous granules, which shows that the basal cells are able differentiate to mucous cells (Fig. 14).

Discussion

Pharynx of *Barbus Sharpeyi* has special form of pharyngeal teeth. Which are suitable for break up of the cellulose cells of the plants. Winfield and Nelson (1991) has reported the non-existence of stomach and absence of effective chemical activity in this fish. Thses finding confirms that *Barbus Sharpyi* are more adapted to



herbivorous feeding. The mucosal epithelium had deep and branched crypts with their bases terminating into mucous acini. Similar structure has been reported in the pharynx of the Rainbow Trout and Sparis Aurata fishes (Takashi, 1982; Cataldi, et al., 1987).

The taste buds were demonstrable in the entire length of the pharynx. (Amin et al., 1992, William and Joseph, 1983) observed taste buds in pharynx of Salmonid. The muscularis mucosa was absent like other species of fish (Takashi, 1982).

The tunica muscularis was striated type and comprised of thick internal (longitudinal) and thin external (circular) layers. The muscularis tissue in Rainbow Trout is striated but its thickness and arrangement of layers are completely reverse (Takashi, 1982). Oesophagus of Barbus Sharepyi, like that of other species of cyprinidea family (Winfield, J. and Melson, S. 1991) and of Tenulosa ictalurus Punctatus fishes is a short, muscular tube (Jafri, 1987, Sis, et al 1979), which connected the pharynx to the intestinal bulb. Two strong sphincters separated it from pharynx and the intestinal bulb. Long primary and secondary folds are also seen in Seriola Drunerili, Angelshark (Grau et al., 1992; Kobegenova, 1993). The mucosal folds permit the swallowing of big baits (Junger et al., 1989; Amin et al., 1992). The opening of swim bladder (Pneumatic Duct) is in the terminal portion of the oesophagus, similar to the Physostom fish (Carl, 1987; Takashi, 1982). The epithelium is like in Ictalurus Punctatus and Tenuolosa llisha which has a few goblet cells (Sis et al., 1979; Jafri and Shaikh, 1987; Carl, 1979). Only a few taste buds were observed in the apex of the primary mucosal folds of the cranial portion of the oesophagus. Cataldi et al., (1987) observed taste buds in the cranial portion of oesophagus in Sparus Aurata and Ictalurus Punctatus. Smith (1969) observed the taste buds in the entire length of the Oesophagus in Galeichthys Felies. The epithelium invaginated to lamina propria and modified into simple mucous acini. No mucous cells were observed in the lamina propria of oesophagus of Ictalurus Punctatus by Sis and et al (1979). Carl (1979), Jafri and Shaikh (1987) reported the presence of mucous glands in the oesophagus. Seromucous acini were seen at the point of opening of pneumatic duct of swim bladder. Amin, (1992); William and Joseph, (1983) observed the serous glands at this point Salmonid. The tunica muscularis was very thick and striated type. Which was arranged in thin internal (longitudinal) layer and thick external (circular) layer. Similar was reported in the Tenuolosa llisha, Ictalurus Punctatus and Catshark (Jafri and Shaikh, 1987; Sis et al., 1979; Kobegenova, 1993). Oesophagus musculature of Salmonid consisted of thin internal (circular) layer and thick external (longitudinal) layer (Amin, 1992; William and Joseph, 1983; Takashi, 1982).

The thickness of the longitudinal muscle layer of Barbus Sharpyi like Ictalurus Punctatus towards to terminal portion. Smooth muscle cells were found scattered at the point of its connection to the intestinal bulb, where the arrangement of cellular muscle layers was reversed (Sis et al., 1979). Muscularis mucosa, stratum compectum and stratum granulosum were absent in oesophagus of Barbus Sharpyi. Kobegenova, (1993) reported the existence of muscularis mucosa in Catshark, Angelshark and Chimera.

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مطالعه میکروسکوپ نوری و الکترونی حلق و مری ماهی بنی

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مطالعه ماکروسکوپی حلق و مری ماهی بنی نشان داد که حلق اندامی قیفی شکل با میانگین طول $1/27 \pm 0/29$ سانتیمتر می باشد. در مدخل و اطراف آن دو استخوان حلقی که بر روی هر یک سه ردیف دندان حلقی با آرایش $5 \cdot 3 \cdot 0 \cdot 2 - 5 \cdot 3 \cdot 0 \cdot 5$ قرار دارد. مری ماهی بین نیز اندامی عضلانی کوتاهی با میانگین طول $0/02 \pm 0/12$ سانتیمتر بوده و دارای دو اسفکتر قدامی (حلقی - مرئی) و خلفی (مرئی - حباب رودهای) می باشد. در انتهای خلفی مری مجرای کیسه شنا باز می شود. مطالعات میکروسکوپی نشان داد که مخاط حلق دارای کریتهای عمیق و مخاط مری دارای چینهای طولی اولیه و ثانویه می باشد. بافت پوششی مخاط حلق و مری از نوع سنگفرشی مطبق شاخی نشده است که سلولهای موکوسی، سلولهای جامی شکل و جوانه های چشایی در آن مشاهده گردید. غدد موجود در دیواره حلق و مری از نوع آسینی منشعب موکوسی که در عمق و جوانب کریتهها قرار دارند. غدد مری در ناحیه انتهایی و در محل باز شدن مجرای کیسه شنا از نوع سروموکوسی می باشند. طبقه عضلانی حلق و مری از نوع مخطط و در جهت طولی (داخلی) و حلقوی (خارجی) آرایش دارند. مطالعات الکترون میکروسکوپی نشان داد سلولهای غدد موکوسی حلق و مری سلولهای استوانه ای بلندی هستند که اعظم سلول توسط گرانولهای ترشحی بزرگ روشنی اشغال شده است. در زیر سلولهای موکوسی، سلولهای قاعده ای قرار دارند. همچنین در مطالعات میکروسکوپ الکترونی، در رأس سلولهای قاعده های تجمعی از مواد موکوسی مشاهده گردید. بنظر می رسد که این سلولها می توانند به سلولهای ترشحی موکوسی متمایز شوند. در سطح سلولهای موکوسی آسینی ها، سلولهای سنگفرشی نیز قرار دارند.

واژه های کلیدی: بافت شناسی، میکروسکوپ نوری و الکترونی، حلق، مری

