CHAPTER THREE

HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON MUCOSUBSTANCES IN THE CARDIAC AND PYLORIC STOMACH OF FISHES AND DISCUSSION
The survey of the existing literature revealed that less attention has been paid to the gastrointestinal tract of the sub-mammalian vertebrates. This is particularly true for the stomach of fishes. Some of the earlier investigators studied only the histology of the stomach in few fishes and there is scanty literature on the nature of mucosubstances present in the various histological sites of the gastric mucosa such as surface epithelial cells, foveolar cells and glands in the pyloric region. The glands in the cardiac stomach have been reported to contain only oxynticopeptic cells and the pyloric glands contain mainly the mucous cells. In this regard there exists scanty literature on the endocrine cells in the gastric mucosa of the fishes. Therefore, an extensive research project has been undertaken to augment the information on the stomach of fishes, wherein even the histology is not known. The present contribution deals with the stomach of three fishes viz. *O. brunneu* (Dori), *R.kangurata* (Mackerel) and *C.gachua* (Murrel).

**OBSERVATIONS**

**Cardiac Stomach**

**Histological Observations**

The stomach could not be demarcated externally from the esophagus. It could be divided histologically into cardiac stomach and pyloric stomach. H-E stained preparations of the cardiac stomach in dori (Fig. 1), mackerel (Fig. 9) and murrel (Fig.17) revealed that the mucosa was highly folded and the longitudinal folds were the rugae. The rugae were broad and short in mackerel than the dori and murrel. The gastric wall consisted of four typical layers viz. mucosa, submucosa, muscularis and serosa. Muscularis mucosa below
The mucosa was noted in murrel. The mucosal epithelium consisted of columnar mucous cells in the surface and in the region of foveolae (gastric pit region) with basally situated nuclei. The simple tubular gastric glands (cardiac glands) consisted of cuboidal to low columnar cells with basally situated nuclei and these were similar to the oxynticopeptic cells described in the other fishes. The mucous neck cells were found to be absent in the cardiac glands in all the three fishes. The gastric glands opened into the bottom of each pit or between the foveolae. In all these type of cells the nuclei were stained blue and cytoplasm pink. The surface mucous cells, foveolar cells appeared slight or moderate blue in M-T staining except foveolar cells in mackerel stained orange. With M-T staining the oxynticopeptic cells were colored orange in all the fishes, and some cells in the glands generally at the basal regions (probably the endocrine cells) appeared deep red. The endocrine cells are known to possess fuschinophilic granules and in M-T staining technique, one of the dye (or stain) is acid fuchsin. The nuclei in various types of cells stained deep red.

The endocrine cells were also confirmed by some special staining procedures which specifically or selectively stain only the endocrine cells. The endocrine cells in the cardiac glands were stained brownish black with Fontana staining procedure and blue-black with lead hematoxylin staining procedure. The endocrine cells were found mainly in the lower half of the cardiac glands. In both these staining procedures, the staining was granular in nature. The endocrine cells were oval or spherical with central nuclei. These cells were numerically less than the oxynticopeptic cells. Two other
methods were also employed, wherein also some of the endocrine cells were selectively stained. With bromophenol blue staining, some of the endocrine cells appeared blue (Fig. 4). Luxol fast blue method also stained some endocrine cells deep blue (Fig. 20). In these staining procedures, the cytoplasm of the endocrine cells was stained homogenously. It is important to control the timing of staining in these two staining procedures for selective staining of the endocrine cells, otherwise other cells in the cardiac glands i.e. oxynticopeptic cells and the mast cells in the submucosa are also stained.

**Histochemical Observations**

The histochemical results on the cardiac stomach are recorded in Table 2 and the distribution of mucosubstances is shown in photomicrographs (dori - Figs. 2, 3; mackerel - Figs. 10-12; murrel - Figs. 18, 19). The following is a brief summary of the results obtained with the histochemical techniques and conclusions drawn from the observations.

**Surface mucous cells**

The surface mucous cells in the cardiac stomach of dori and few cells in mackerel exhibited an intense PAS reactivity (Figs. 2, 10) which was resistant to α-amylase digestion but could slightly be blocked by phenylhydrazine pretreatment. These initial results indicated absence of glycogen but presence of neutral mucosubstances in these cells. The presence of neutral mucosubstances was also indicated by purple staining with AB pH 1.0 - PAS, AB pH 2.5 - PAS and C.I. - PAS sequential staining procedures and enhanced metachromasia with azure A after induced sulfation.
### TABLE 2: COMPARATIVE HISTOLOGICAL AND HISTOCHEMICAL STAINING REACTIVITIES IN THE CARDIAC AND PYLORIC STOMACH OF FISHES.

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<thead>
<tr>
<th>Histological and Histochemical Staining methods</th>
<th>Cardiac Stomach</th>
<th>Pyloric Stomach</th>
<th>Pyloric glands</th>
<th>Endocrine cells</th>
<th>Pyloric cases in Murell</th>
<th>Doris</th>
<th>Murell</th>
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NOTE: The above table represents the comparative histological and histochemical staining reactivities in the cardiac and pyloric stomach of fishes. The reactions are indicated by '+', with the intensity varying from '+++' to '++'.
CAPTIONS TO FIGURES

Fig. 1 - T.S. of cardiac stomach of *O. brunneus* 
H-E staining X 120.

Fig. 2 - T.S. of cardiac stomach of *O. brunneus* 
PAS staining X 200.

Fig. 3 - T.S. of cardiac stomach of *O. brunneus* 
C.I. staining X 200.

Fig. 4 - T.S. of cardiac stomach of *O. brunneus* 
Bromophenol blue staining X 200.

Fig. 5 - T.S. of pyloric stomach of *O. brunneus* 
H-E staining X 200.

Fig. 6 - T.S. of pyloric stomach of *O. brunneus* 
PAS staining X 200.

Fig. 7 - T.S. of pyloric stomach of *O. brunneus* 
C.I. staining X 120.

Fig. 8 - T.S. of pyloric stomach of *O. brunneus* 
Bromophenol blue staining X 200.

ABBREVIATIONS

E = Endocrine cells  
F = Foveolar cells  
O = Oxynticopeptic cells  
PG = Pyloric glands  
S = Surface mucous cells
CAPTIONS TO FIGURES

Fig. 9 T.S. of cardiac stomach of R. kanguurta 
H-E staining X 200.

Fig.10 T.S. of cardiac stomach of R. kanguurta 
PAS staining X 120.

Fig.11 T.S. of cardiac stomach of R. kanguurta 
AB pH 2.5 staining X 200.

Fig.12 T.S. of cardiac stomach of R. kanguurta 
AF staining X 120.

Fig.13 T.S. of pyloric stomach of R. kanguurta 
M-T staining X 200.

Fig.14 T.S. of pyloric stomach of R. kanguurta 
PAS staining X 200.

Fig.15 T.S. of pyloric stomach of R. kanguurta 
AB pH 1.0 staining X 200.

Fig.16 T.S. of pyloric stomach of R. kanguurta 
Lucol fast blue staining X 200.

ABBREVIATIONS

CE = Columnar epithelial cells    E = Endocrine cells
F = Foveolar cells        O = Oxynticopeptic cells
PG = Pyloric glands        S = Surface mucous cells
S_1 = Surface mucous cells with neutral-, sulfo- and sielomucins
S_2 = Surface mucous cells with only neutral mucins.
CAPTIONS TO FIGURES

Fig.17  T.S. of cardiac stomach of C. gachua
        H-E staining X 200.

Fig.18  T.S. of cardiac stomach of C. gachua
        PAS staining X 200.

Fig.19  T.S. of cardiac stomach of C. gachua
        AB pH 1.0 staining X 200.

Fig.20  T.S. of cardiac stomach of C. gachua
        Luxol fast blue staining X 400.

Fig.21  T.S. of pyloric stomach of C. gachua
        H-E staining X 200.

Fig.22  T.S. of pyloric stomach of C. gachua
        PAS staining X 180.

Fig.23  T.S. of pyloric stomach of C. gachua
        AB pH 1.0 staining X 200.

Fig.24  T.S. of pyloric stomach of C. gachua
        Bromophenol blue staining X 400.

Fig.25  T.S. of pyloric caecum of C. gachua
        PAS staining X 200.

ABBREVIATIONS

A = Absorptive cells
F = Foveolar cells
F_2 = Foveolar cells
(O) = Oxynticopeptic cells
S = Surface mucous cells
E = Endocrine cells
F_1 = Foveolar cells
G = Goblet cells
PG = Pyloric glands

(Upper) with only neutral mucins
(lower) with neutral and sulfomucins
Moreover, these cells exhibited weak alcianophilia at pH 1.0 and moderate alcianophilia at pH 2.5 (Fig. 11), thus indicating the presence of both sulfomucins and carboxymucins in these cells. The sulfomucins were further identified by purple staining with AF (Fig. 12), blue-purple staining with AF-AB pH 2.5 sequence, weak metachromasia with azure A at low pH (pH 1.5), persistent alcianophilic staining in CEC staining procedure at and above 0.2 M Mg²⁺ concentration and elimination of alcianophilia by active methylation which could not be restored completely following subsequent saponification.

The presence of carboxymucins in these cells was substantiated by C.I. reactivity (Fig. 3) at the same grade as that in the AB pH 2.5 procedure, blue-purple staining with AF - AB pH 2.5 sequence, enhanced metachromasia with azure A at pH 3.0 and above and blockade of alcianophilia by mild and active methylations which was restored partly after subsequent saponification. The carboxymucins were further identified in these cells as acid hydrolysis partly reduced their alcianophilia. These results thus indicated that the surface mucous cells in dori and few cells in mackerel contained neutral-, sulfo- and sialomucins.

On the other hand the surface mucous cells in murrel and majority of cells in mackerel reacted intensely towards PAS (Figs. 10, 18). Their PAS reactivity was resistant to α-amylase digestion but was resistant to prior phenylhydrazine treatment. These cells remain unstained with AB pH 1.0 (Fig. 19), AB pH 2.5 (Fig. 11), C.I. and AF and exhibited only orthochromatic blue staining with azure A at higher pH levels. Moreover, these cells exhibited metachromatic pink staining after sulfation. There was no alcianophilia in these...
cells even after pepsin digestion. The aforementioned observations indicated the presence of only neutral mucosubstances in the surface mucous cells in murrel and majority of cells in mackerel.

**Foveolar Cells**

The foveolar cells in the gastric mucosa in dori (Figs. 2, 3) and superficial foveolar cells in murrel (Figs. 18, 19) exhibited only an intense PAS reactivity and contained only neutral mucosubstances in them. This conclusion was drawn from the similarity of their staining reactivities with other histochemical procedures as described for the surface mucous cells in murrel and majority of cells in mackerel.

The foveolar cells in the gastric mucosa of mackerel on the other hand contained neutral mucosubstances (poor) and sialomucins (predominant). The neutral mucosubstances were characterized by slight reduction in their PAS reactivity than normal PAS (Fig. 10), purple-blue combined coloration with AB pH 1.0-PAS, AB pH 2.5-PAS, C.I.-PAS and enhanced metachromasia with azure A after induced sulfation. The sialomucins were characterized from their weak to moderate alcianophilia at pH 2.5 (Fig. 11) (but not at pH 1.0), weak to moderate C.I. reactivity, only blue staining with AF-AB pH 2.5 sequence, metachromasia with azure A at and above pH 3.0, reversible blockade of their alcianophilia in mild and active methylations and subsequent saponification and loss of alcianophilia following acid hydrolysis.

The foveolar cells in the basal region in the gastric mucosa of murrel reacted intensely towards PAS (Fig. 18). Their PAS reactivity was resistant to α-amylase digestion but could partly be
blocked by prior phenylhydrazine treatment. These results indicated absence of glycogen but presence of some neutral mucosubstances in them. The neutral mucosubstances were also demonstrated in these cells by blue-purple staining with AB pH 1.0 - PAS, AB pH 2.5 - PAS and C.I. - PAS sequences and enhancement in metachromatic pink staining with azure A after sulfation.

Moreover, these cells exhibited poor to week alcianophilia at pH 1.0 (Fig. 19) but their alcianophilia was not enhanced at pH 2.5, which indicated the presence of sulfomucins. The presence of sulfomucins was also inferred from their only purple staining with AF-AB pH 2.5 sequence, metachromasia with azure A even at pH 1.5, persistent alcianophilic staining in CEC technique at and above 0.2 M Mg++ concentration and irreversible loss of alcianophilia in mild and active methylation-saponification procedures. These results indicated the presence of neutral mucosubstances and sulfomucins in the basal foveolar cells in murrel.

**Oxynticopeptic cells**

The oxynticopeptic cells in the cardiac glands of dori (Fig. 2) and mackerel (Fig. 10) contained only traces of neutral mucosubstances. This conclusion was based on their poor PAS reactivity. The rest of the staining reactivities resembled to that of surface mucous cells in murrel and majority of surface mucous cells in mackerel except poor intensity with PAS staining. The oxynticopeptic cells in the gastric mucosa in murrel did not contain any mucosubstances as they remain unstained with all the histochemical procedures including PAS (Fig. 18).

**Pyloric stomach**

There was no external demarcation between the cardiac stomach
and pyloric stomach. These two regions were distinguished in histological stained preparations. The broad and short rugae or primary folds were found in the pyloric stomach of dori (Fig. 5). Such primary folds were very few, hardly one or two in murrel (Fig. 21). H-E stained preparations revealed variable number of projecting secondary folds of the pyloric mucosa in dori. The pyloric mucosa was folded in these fishes. The epithelium lining the mucosa at surface and foveolar regions consisted of mucous cells. Pyloric glands were observed below the foveolar region in dori (Fig. 5) and mackerel. The pyloric glands were few, rounded in appearance and opened at the pit region between the folds at the base of foveolae. The pyloric glands were not observed in murrel (Fig. 21). The mucosa was thinner at and near the pyloric sphincter in all the fishes. The nuclei in all the cell types such as surface mucous cells, foveolar cells and pyloric glands stained blue and cytoplasm pink with H-E staining procedure. M-T staining (Fig. 13) revealed dark red nuclei and blue staining in supranuclear area in the surface mucous cells and foveolar cells. The cells in the pyloric glands of dori and mackerel appeared orange. Occasionally deep red or orange red cells were observed in the pyloric glands in dori and mackerel and at the base or basal lateral regions of foveolae in murrel. These cells were considered as endocrine cells. The endocrine cells were further demonstrated by brown-black staining with Fontana (Silver impregnation) method, blue-black staining with lead hematoxylin and blue coloration with bromophenol blue (Figs. 8, 24) and luxol fast blue (Fig. 16) staining procedures. The endocrine cells were smaller and spherical in shape with central nuclei.
Finger-like projections were found from the distal region of pyloric stomach near the junction between the pyloric stomach and duodenum in murrel. Such outgrowths are called as pyloric caeca. Histologically they resembled intestine as the mucosal villi projected into the lumen (Fig. 25). The mucosal epithelium of villi was single layered and contained columnar absorptive cells and goblet cells between them. The submucosal connective tissue extended in each villus forming the core of the villus. The pyloric caeca were not observed in other two fishes under present investigation.

Histochemical Observations

The histochemical results on pyloric stomach are recorded in Table 2 and the distribution of mucosubstances is shown in photomicrographs (dori - Figs. 6, 7; mackerel - Figs. 14, 15; murrel - Figs. 22, 23; pyloric caecum in murrel - Fig. 25). The results requiring further consideration and conclusions drawn are presented hereafter.

Surface mucous cells

All the surface mucous cells in the pyloric stomach of dori, murrel and majority of cells in mackerel exhibited an intense PAS reactivity (Figs. 6, 14, 22) and no basophilia with AB pH 1.0 (Figs. 15, 23), AB pH 2.5, C.I. (Fig. 7) and AF. The histochemical results in these cells were identical to that described for the surface mucous cells in the cardiac stomach of dori and majority of cells in mackerel. Therefore it was concluded that the surface mucous cells in dori, murrel and most of the cells in mackerel contained only neutral mucosubstances.

Numerically few surface mucous cells in the pyloric stomach of
mackerel exhibited different staining reactivities. These cells exhibited an intense PAS staining (Fig. 14) which remain unaltered following α-amylase digestion and phenylhydrazine pretreatment. These results indicated absence of glycogen and neutral mucosubstances in these cells. Moreover these cells showed an intense blue staining with AB at pH 1.0 which was not enhanced at pH 2.5 and even in AB pH 1.0 - PAS and AB pH 2.5 - PAS sequential staining procedures, there was only blue staining. These results indicated the presence of only sulfomucins. This conclusion was also substantiated by only purple staining with AF alone or with AB pH 2.5 step afterwards, metachromasia with azure A at low pH (pH 1.5), presence of alcianophilia in CEC technique at and above 0.2 M Mg²⁺ concentration and elimination of their alcianophilia by active methylation which could not be restored following saponification.

Foveolar cells

The foveolar cells in the pyloric mucosa of dori (Figs. 6, 7), murrel (Figs. 22, 23) and mackerel (Figs. 14, 15) resembled in their histochemical reactivities to those exhibited by foveolar cells in the cardiac stomach of dori, murrel and majority of cells in mackerel. Therefore it was concluded that pyloric foveolar cells in all the fishes contain only neutral mucosubstances (predominant in dori and murrel and less in mackerel). The only difference was that these cells stained intensely with PAS in dori and murrel but weakly in mackerel.

Pyloric glands

The cells in the pyloric glands of dori (Figs. 6, 7) resembled to the foveolar cells in dori in histochemical staining reactivities.
Therefore it was concluded that these pyloric glands also elaborate predominant neutral mucosubstances. The pyloric glands in mackerel also elaborate only neutral mucosubstances as the foveolar cells in this fish. This conclusion was based on weak staining with PAS only and weak metachromasia with azure A only after sulfation.

**Pyloric caeca**

The columnar epithelial absorptive cells in the pyloric caeca of murrel did not react with any of the histochemical staining procedures except the brush border (Fig. 25).

The goblet cells in the epithelium of pyloric caeca exhibited an intense PAS reactivity (Fig. 25) which was resistant to α-amylase digestion and phenylhydrazine pretreatment. These observations revealed the absence of glycogen and neutral mucosubstances. The goblet cells showed moderate alcianophilia at pH 1.0 and an intense alcianophilia at pH 2.5. These results indicated the presence of sulfomucins (predominant) and sialomucins (poor) in the goblet cells.

The presence of sulfomucins in these cells was further supported by blue-purple staining with AF-AB pH 2.5 sequence, moderate metachromasia with azure A at pH 1.5, persistent alcianophilic staining in CEC procedure at and above 0.2 M Mg^{++} concentration (upto 0.5 M) and loss of alcianophilia after active methylation which could not be restored completely following saponification. The sialomucins in these cells were characterised by blue-purple staining with AF-AB pH 2.5 sequence, enhanced metachromasia with azure A at pH 3.0 and above, only partial restoration of alcianophilia by active methylation-saponification procedure and partial reduction in alcianophilia at pH 2.5 after acid hydrolysis. Thus, the histochemical results
revealed that the goblet cells in the pyloric caeca of murrel secrete sulfomucins (predominant) and sialomucins (poor).

DISCUSSION

The present investigation was undertaken with a view to augment the information on the gastric mucosa of vertebrates which are not investigated. The present chapter deals with the histology (cell types) of the stomach in fishes, distribution of mucosubstances in various cell types, species diversity, if any, relationship between mucosubstances and feeding habits, if any and to compare the results obtained in the present investigation with the existing data to find out similarities or differences, if any, among fishes. The phylogenetic relationships of the gastric structure among vertebrates will be discussed separately along with general discussion.

The stomach was present in all the three fishes under present investigation, although it is not demarcated externally from the esophagus. Cardiac stomach and pyloric stomach also could not be distinguished externally. On the other hand, from the histological observations it is possible to distinguish cardiac stomach from esophagus and pyloric stomach from cardiac stomach. There are transitional structures at each junctions and one region does not abruptly change into the other. This is also reported by Khanna (1977), Reifel and Travill (1978) and many others.

On the other hand all the fishes may not possess a true stomach and it is absent in number of fishes. In such fishes the anterior part of the intestine is swollen to form a sac behind esophagus. This structure serves for the storage of food and is called as intestinal bulb. The intestinal swelling or intestinal bulb is present in
Labeo rohita, L. gonius, Cirrllina mrigala, Tor tor, Catla catla, Puntius sopore etc. The absence of true stomach is a special feature of Cyprinids, but presence or absence of stomach is not related to the feeding habits of the fish. The stomach is also absent in Holocephali and Dipnoi (Khanna, 1977). Besides Cyprinids, some other fishes like Scomberesox, Xenentodon and Hippocampus are also without stomach.

The gastric glands are not present in the intestinal bulb and its mucosa resembles closely with that of the intestine. Absorptive cells and mucus secreting cells are the two main types of cells in the mucosal epithelium of the intestinal bulb. In the present investigation pyloric caeca were observed in one fish (murrel), the structure of which resembles the intestinal bulb or the intestine. A question arises here that "whether the pyloric caeca present in some fishes represent the remnant of intestinal bulb (vestigial structure) or the pyloric caeca serve to increase the surface area for absorption?"

Several views have been proposed to explain why stomach is absent in some fishes. According to Barrington (1957) absence of stomach in some teleosts might have been brought about by neoteny. Barrington (1942, 1957) suggested that the absence of stomach in Cyclostomata as a primitive feature and the appearance of a stomach in higher fishes may be correlated with the establishment of macrophagous feeding, which was rendered possible by the evolution of jaws.

The view that the teleostean stomach originated for food storage is supported by the existance of proximal intestinal swelling
in agastric Cyprinids. It is possible that glandless stomach reported by Ishida (1935) might have evolved from the intestinal bulb and then the secretion of acid enzymes followed. Barrington (1942, 1957) also regarded that because stomachs are present in primitive fishes, their absence in Cyprinids and other fishes may be a secondary condition. This is evidenced by the fact that the sub-order Ostariophysi, which contain agastric Cyprinids, appear to be derived from more primitive Protacanthopterygians (Romer, 1966) which have stomachs. On the other hand Young (1950) opined that fish have followed their own lines of specialization, often with confusing amount of parallel evolution and therefore have evolved features which are unknown in higher forms, or if known, have appeared quite independently.

**Cardiac Stomach**

The stomach is not demarcated externally from the esophagus in the three fishes investigated. From the histological structures it was possible to distinguish the stomach from esophagus by difference in the mucosal folds and thickness of the glandular mucosa. The histology of the gastric mucosa in the three fishes is simpler than the higher vertebrates in that the mucosal epithelium consisted of columnar mucous cells (also referred to them as goblet cells by others), in the surface and foveolae (pit regions) and presence of glands with only one type of cells (oxynticopeptic cells, presumed to secrete both enzyme-pepsinogen and HCl). More or less similar histology of the cardiac (corpus or body) stomach has been described for other fishes (Pasha, 1964; Jirge, 1970; Sastry, 1973; Reifel and Travill, 1978; Gas and Noaillac-Dedpeyre, 1978; Sis et al., 1979;
Suganuma et al., 1981a; Jadhav, 1985). Sastry (1973) found absence of stomach in Cirrhinus but its presence in Clarias. Most of the investigators consider that the lining epithelium of mucous cells similar on the surface of the folds and in the pit region (foveolar region) identical but Reifel and Travill (1978) found differences in these cells in some fishes based on histochemical reactivities which characterize their type/s of mucosubstances. The present investigation also supports the above view that the mucosubstances elaborated by the surface mucous cells and foveolar cells may differ in some fishes.

Numerous gastric glands of simple tubular type are present below the epithelium in the gastric mucosa of the three fishes under present investigation. In the gastric glands (cardiac) of these fishes, the mucous neck cells are absent. The mucous neck cells are present in amphibians, reptiles and mammals. Similar histological and histochemical observations by other investigators also revealed the absence of mucous neck cells in the gastric glands of other fishes (Barrington, 1957; Reifel and Travill, 1978; Suganuma et al., 1981a; Jadhav, 1985).

Edinger (1877) first stated that gastric glands of teleosteans are not differentiated into parietal and chief cells as are those of mammalian stomach. All subsequent workers have confirmed this finding. On the basis of light microscopic studies, it is not possible to correlate the gastric gland cells of teleosts with those found in mammalian stomachs, although morphologically they may more closely resemble the chief cells which are enzyme secreting cells of the stomach. The question then arises that if cells comparable to the HCl secreting parietal cells are absent, what is the source of HCl in
Although Stirling (1884) considered the possibility that the superficial mucous cells secrete both acid and mucus, it would appear more likely that gastric glands of teleosts more closely resemble the secretory cells in the gastric glands of amphibians which have the capacity to secrete both HCl and enzyme (pepsinogen) (Sedar, 1961a, b, 1962).

It was found that the cytoplasmic granularity of the amphibian gastric gland cells (oxynticopeptic cells) is due to numerous mitochondria and the zymogen granules seen in electron microscopic observations. The structures of mitochondria resemble that described in mammalian parietal cells, while the zymogen granules resemble those of mammalian chief cells. The granularity of teleostean gastric gland cells is also possibly due to the parietal cell type mitochondria and chief cell type zymogen granules. Recently Connes et al. (1983) reported that the glands of the sea bass, *Dicentrarchus labrax* are mainly composed of cell showing ultrastructural characteristics of both main cells (Chief cells) and oxyntic cells of mammalian stomach. These are called as oxynticopeptic cells. The polymorphous intracytoplasmic canaliculi, still unknown in lower vertebrates are present. Obviously these cells secrete both pepsin-like enzyme and HCl.

Ezeasor (1981) also described the fine structure of surface mucous cells, oxyntic cells and endocrine cells in the gastric mucosa of the rainbow trout, *Salmo gairdneri*. The single type of gastric gland cells producing both pepsinogen (or pepsin-like enzyme) and HCl are also reported in several fishes (Barrington, 1957; Pasha, 1964; Khanna, 1977; Gas and Noillac-Dedpeyre, 1978; Jadhav, 1985). Reifel and Travill (1978) described that the simple tubular glands in the gastric mucosae of eight different fishes are simple and tubular and
open at the bottom of gastric pit. The component cells of the glands are of one type only and contain eosinophilic granules. In the present investigation also single type of cells were observed in the gastric glands of three fishes and these resemble to the oxynticopeptic cells in other fishes. In the lower vertebrates the same cell which secretes both pepsinogen and HCl is variously been known as oxyntic cells, chief cells, zymogen cells, oxynticopeptic cells etc. The term oxynticopeptic is now widely used to designate such cells in the gastric glands of lower vertebrates.

The present investigation also revealed the presence of endocrine cells in the gastric glands of three fishes. The occurrence of three kinds of gut endocrine cells in some cartilaginous fish species has been reported by Gabe and Martoja (1972). Ezeasor (1981) described the ultrastructure of endocrine cells in the stomach of S. gairdneri. In the present investigation also endocrine cells were observed in the gastric glands in the cardiac region. Most of the cells were identified by Fontana (Silver impregnation) method, lead-hematoxylin method and few cells were also stained selectively by bromophenol blue and luxol fast blue methods. Based only on light microscopic observations these cells cannot be classified (classification of endocrine cells is described in chapter-one). Recently Cimini (1985) studied the endocrine cells in the gastric mucosa of two elasmobranch fishes by light and electron microscope by silver impregnation method. He identified five cell types in the fundic mucosa, four of which are of "open type". All the cells show polymorphic granules of variable size except those of type V cell, which are round in shape and smaller. He further reported that no
functional classification or analogies with other vertebrate gastric endocrine cells were attempted as these would be too speculative on the basis of ultrastructural characters only. Recently Fujita and Kobayashi (1977) in a review on structure and function of gut endocrine cells reported that our knowledge of the gut endocrine elements in bony fishes is very meager.

The results obtained with histochemical staining methods for the characterization and distribution of mucosubstances in the gastric mucosa of the cardiac stomach in three fishes revealed a heterogeneous distribution of neutral-, sulfo- and sialomucins.

The surface mucous cells in dori and few cells in mackerel contain neutral-, sulfo- and sialomucins, whereas only neutral mucosubstances in murrel and few cells in mackerel. The foveolar cells contain only neutral mucosubstances in dori and superficial cells in murrel; neutral- and sialomucins in mackerel and neutral- and sulfo-mucins in few basal (deeper) foveolar cells in murrel. The oxyntico-peptic cells in the gastric glands in dori and mackerel contain poor quantities of only neutral mucosubstances.

When the results obtained in the present investigation are compared with the existing data, some similarities and differences can be noted for the distribution of mucosubstances in the different cell types of the gastric mucosa of the fishes. Bucke (1971) reported on PAS and AB reactive epithelial cells in the stomach of *E. lucius*. The surface mucous cells (referred to them as goblet cells by some investigators) contain only neutral mucosubstances in snakehead fish (Suganuma et al., 1981a), *C. magur* (Jadhav, 1985), *I. nebulosus*, *A. rupestris*, *L. macrochirus*, *M. salmoides*, *P. nigromaculatus*,...
and *P. flaviscens* (Reifel and Travill, 1978). In the present investigation these cells in the cardiac stomach also contained only neutral mucosubstances in murrel and few cells in mackerel. These cells contain only sialomucins in *G. batrachus* (Shafi, 1974), sialomucins in shark and rainbow trout (Suganuma *et al.*, 1981a) and *E. lucius* and *E. americanus* (Reifel and Travill, 1978). Only sulfomucin or only sialomucin containing surface mucous cells are absent in the stomach of the three fishes investigated. Moreover, Jadhav (1985) found neutral- and sulfomucins in the surface mucous (goblet cells) in *I. mossambica*. In addition he found simple columnar cells between these goblet cells which contain traces of neutral mucosubstances. In the present investigation neutral-, sulfomucins and sialomucins were identified in the surface mucous cells in the stomach of dori and few cells in mackerel.

The cells in the pit region (foveolar cells) of the stomach also show variations in their mucosubstance content in different fishes. The cells in the pit region of the stomach have been reported to contain neutral mucosubstances in *I. nebulosus* and *M. salmoides*; more acidic mucins in *E. lucius*; sialomucins in *E. americanus*; weakly acid sulfated mucins in *P. nigromaculatus* and a mixture of neutral mucosubstances and weakly acidic sulfated mucins in *A. rupestris* and *L. macrochirus* (Reifel and Travill, 1978). The present investigation also shows a heterogeneity of mucosubstances in the foveolar cells of the gastric mucosa which contain neutral mucosubstances in dori and superficial cells in murrel; neutral- and sialomucins in mackerel and neutral- and sulfomucins in some basal or deeper cells in murrel.

The present investigation also revealed the presence of neutral mucosubstances (traces) in the oxynticopeptic cells in the gastric
glands of dori and mackerel. Earlier, Jirge (1970) also found neutral mucosubstances in the gastric glands of *T. mossambica*.

The aforementioned comparative account also reveals the species diversity in the type/s of mucosubstances in the surface mucous cells, foveolar or pit cells and oxynticopeptic cells in the glands. Not only this, but the species diversity also exists with regard to the presence or absence of stomach (discussed earlier in detail), of endocrine cells, type of cells present in foveoli and surface epithelial cells. For example, Jadhav (1985) observed simple columnar epithelial cells and mucous cells (goblet cells) in the surface epithelium of gastric mucosa in *T. mossambica*. The present investigation also revealed two types of surface mucous cells in the gastric mucosa of mackerel and histochemically different superficial and basal, foveolar cells in the murrel. The reasons for such species diversity are not known at present.

**Pyloric stomach**

The histology of the pyloric stomach in the fishes investigated was very simple. The mucosa is folded and covered with columnar surface mucous cells. The foveolar region is also lined by mucous type of cells. The pyloric glands below the epithelium and foveolae containing only mucous type of cells are present in dori and mackerel. The pyloric glands are absent in murrel. Sis *et al.* (1979) observed only columnar epithelial cells in the pylorus of *I. punctatus*, the pyloric glands being absent. Absence of pyloric glands has also been reported in *M. cyprinoides* (Pasha, 1964), eight species of fishes (Reifel and Travill, 1978) and seven species of fishes (Suganuma *et al.*, 1981a). The presence of pyloric glands has been reported in fishes
(Patt and Patt, 1969; Jadhav, 1985). Jadhav (1985) also reported that the mucosal epithelium consists of only goblet cells in *C. magur* but columnar epithelial and goblet cells in *T. mossambica*. The present studies have also demonstrated endocrine cells in the pyloric glands of two fishes and at the base of epithelium in murrel. With four histological staining procedures viz. Fontana staining, lead-hematoxylin, bromophenol blue and luxol fast blue the endocrine cells in the pyloric gastric mucosa were identified. Based only on light microscopic studies, it is not possible to classify them, although some of the cell types have been identified in the pyloric stomach particularly of mammals by electron microscopic studies.

The histochemical observations in the present investigation revealed presence of neutral mucosubstances (predominant) in the surface mucous cells in the pyloric stomach of dori, murrel and most of the cells in mackerel. On the other hand, few surface mucous cells in mackerel elaborate only sulfomucins. The foveolar cells in all the fishes contain only neutral mucosubstances, although predominant in dori and murrel and poor quantities in mackerel. The pyloric glands present in dori and mackerel elaborate and secrete only neutral mucosubstances, predominant in dori and poor quantities in mackerel.

When the results obtained in the present investigation are compared with that of the existing literature, some similarities and differences can be noted. The columnar epithelial cells containing poor quantities of neutral mucosubstances reported in *T. mossambica* (Jadhav, 1985) have not been reported in other fishes. The surface mucous cells have been reported to elaborate only neutral mucosub-
stances in *C. magur* (Jadhav, 1985) and *I. nebulosus*, *P. flaviscens*, and *M. salmoides* (Reifel and Travill, 1978), sialomucins in *E. lucius*; sialidase resistant sialomucins in *A. rupestris*, *L. macrochirus* and *P. nigromaculatus* and sialomucins and sulfomucins in *E. americanus*. On the other hand Jadhav (1985) identified neutral- and sulfomucins in the surface mucous (goblet) cells in the pyloric stomach of *T. mossambica*.

Although most of the investigators found absence of the glands in the pyloric region of the stomach in fishes, Jadhav (1985) found glands which contain only neutral mucosubstances. In the present investigation glands were found in the pyloric stomach of dori and mackerel which also elaborate only neutral mucosubstances in variable quantities.

The aforementioned results obtained in the present investigation and the limited histochemical data on the mucosubstances in the various regions of the pyloric stomach also indicate that species diversity exists in relation to the presence or absence of the pyloric glands and the nature of mucosubstances elaborated by the surface mucous cells.

**Pyloric Caeca**

In the present investigation finger-like paired outgrowths were observed at the junction of pyloric stomach and duodenum in one fish only, *C. gachua*. Their histology is similar to the intestine with villi covered by single layer of epithelial cells. Two types of cells are present in the epithelium, columnar absorptive cells and goblet cells. Khanna (1977) described that number of finger-like processes or outgrowths develop from the pylorus or the anterior part
of intestine are called as pyloric caeca or intestinal caeca. They are present in *Notopterus, Channa, Mastacembelus, Hilsa, Herpodon* etc. Their number varies from one to several hundred.

According to Rahimullah (1945), these appendages should be called intestinal caeca and they serve as accessory food reservoirs. Histologically they resemble intestine and probably serve to increase its absorptive area. They are not found in stomachless fishes and have no taxonomic value as they are found in large number of fishes, belonging to widely different families.

The present investigation revealed the presence of sulfomucins (predominant) and sialomucins (poor) in the goblet cells of pyloric caeca in murrel. When the results obtained on mucosubstances in the caecal goblet cells are compared with the intestinal goblet cells some similarities and differences become apparent and the intestinal goblet cell mucosubstances also exhibit species diversity. Bucke (1971) demonstrated PAS and AB reactive intestinal goblet cells in *E. lucius*. Acidic mucopolysaccharides and sulfated mucopolysaccharides were identified in these cells of *C. batrachus* (Shafi, 1974). Kim (1972) reported on the presence of neutral and acidic sulfated mucopolysaccharides in the intestinal goblet cells in seven species of fishes. Reifel and Travill (1979) demonstrated sulfomucins and sialidase resistant sialomucins in the intestinal goblet cells of *A. rupestris, L. macrochirus, M. salmoides, P. nigromaculatus, I. nebulosus, P. flavescens, Notemigonus crysoleucas* and *Primephales promelas*; neutral and sialomucins in *E. lucius* and only sialomucins in *E. americanus*. Jadhav (1985) distinguished two types of intestinal goblet cells in *C. magur and T. mossambica*; the type-I cells contain
only sulfomucins and type-II cells contain neutral mucosubstances and sulfomucins. Thus at a gross level it appears that sulfomucins are secreted by the intestinal cells; sometimes also sialomucins and/or neutral mucosubstances also. In this regard the pyloric caecal goblet cells resemble sulfomucins secreting intestinal goblet cells.

Several functions have been attributed to the pyloric caeca or intestinal caeca. They may serve as accessory food reservoirs or probably serve to increase the absorptive area. It also appears that these may represent rudiments of intestinal bulb of stomachless fishes. The histology of the intestinal bulb in stomachless fishes also resembles intestinal histology. Ziswiler and Farner (1972) also described intestinal caeca of birds and reviewed earlier literature. They described that the intestinal caeca, present as rudiments or lacking in the birds belonging to the families Trochilidae and Apodidae. Such caeca are well developed in herbivorous and omnivorous species and rudimentary in fish feeders. The mucus secreted by the caecal goblet cells may be playing the role of protection of the mucosa. Mitjavilla et al. (1968) and Fox (1979) also attributed protective role to the intestinal mucus in mammalian intestine.