Chapter 5

Discussion
5.1. HISTOCHEMISTRY

Iron in the tissues is mostly present in ferric form. Perl's Prussian blue method (cf. Pearse, 1972) has been employed for the histochemical demonstration of iron in the present study as the said method has been considered as the method of choice for the localization of iron by Pearse (1972). The present observations clearly depict the presence of iron only in the liver of *Schizothorax curvifrons*. The occurrence of iron in the liver of the investigated fish is indicative of the fact that iron is stored in the liver which is utilized for the various physiological activities, as has also been reported by Mitchell (1956). Iron as a component of haemoglobin, myoglobin and cytochromes plays a key role in oxygen transport and cellular oxidation. The absence of iron from the gills probably suggests that they are neither the routes of absorption nor the sites of storage whereas, negative reaction for iron observed in the entire intestinal tract is possibly due to two reasons viz. (i) the amount of iron in the natural diet of the fish is too small to be localized histochemically or (ii) even if absorbed, it is immediately transported to the circulatory system.

Although a few methods are known for the detection of calcium in the tissues (Cameron, 1930; McGee Russel, 1958 and Carr *et al.*, 1961), Dahl's Alizarin red S method (1952) has been adopted for the histochemical localization of calcium in the present investigation. According to Pearse (1972), the method of Dahl (1952) is more sensitive as compared to other methods and chiefly stains calcium deposits. Even Dahl (1952) regards the sensitivity of Alizarin red S method to be greater when used histochemically. Besides, the said method can be conveniently applied at room temperature and is specific for calcium. In the present study, no adhesive was used for mounting the sections because it has been seen by Dahl (1952) that both gelatin and egg albumin caused fixation of the stain in a variable manner.
The present observations reveal the presence of calcium only in the intestinal bulb and intestine of the fish studied. Mitchell (1956) states that the actual site of absorption of calcium is the intestinal villus, which has also been confirmed by the present histochemical study. As the said tissues do not normally synthesize calcium, its presence in these tissues can be explained on the basis that the dissolved minerals in water or mixed with food or in a free state are taken up during the normal course of feeding which get absorbed and are then transported to the blood stream for the maintenance of various metabolic and physiological needs. According to Bykov (1958), the calcium salts are absorbed relatively in small quantities so that this is not attended by any sharp increase in the content of calcium in the blood. The same author further states that the salts of calcium are absorbed best when they are consumed with food. According to him, the absorbed calcium leaves the intestine through blood vessels and lymph spaces as is also evident from the present investigation. Similar observations have been made with respect to the fishes fed artificially on the diet containing calcium (Channa, 1981). Calcium is required for regulating a large number of cellular activities and exerts a regulatory function on blood coagulation and permeability of cell membranes and capillaries.

Proteins are the most abundant biological macromolecules occurring in all cells. Proteins exhibit enormous diversity of biological functions and are the most important final products of the information pathways. All proteins are constructed from the same ubiquitous set of twenty amino acids, covalently linked in characteristic linear sequence. As each of these amino acids has a side chain with distinctive chemical properties, so this group of twenty precursor molecules may be regarded as the alphabets in which the language of protein structure is written.
It has been observed that the gills, entire intestinal tract and the liver of the *Schizothorax curvifrons* react intensely for general proteins adopting Mercury bromophenol blue method (cf. Pearse, 1972). The chloride and the pavement cells of the gills, the mucosal epithelial cells, the entire submucosal region, muscularis, serosa and the hepatocytes are the localized sites. Intense reaction for proteins observed indicates their role in synthetic activities, besides being the prominent cellular constituents (Medeiros *et al.*, 1970; Woodward and Bergeron, 1984 and Abdelmeguid *et al.*, 2002).

In the living tissues, the ingested proteins are acted upon by proteolytic enzymes in order to make the amino acids free for their easy and quick transport through blood for absorption. The free absorbed amino acids in the cells are then covalently linked to synthesize the proteins of desired quality for various metabolic and physiological activities. Tryptophan is an essential aromatic and relatively non-polar (hydrophobic) amino acid of neutral group of amino acids.

Although some specific histochemical reactions are available for the localization of tryptophan (Adams, 1957; Glenner and Lillie, 1957 and Bruemmer and Thomas, 1958), Adams (1957) p-Dimethylaminobenzaldehyde (DMAB)-nitrite method has been employed for the histochemical localization of tryptophan in the present study because of its high specificity (Pearse, 1972). Adams (1957) preferred alcoholic fixatives to those containing formaldehyde. In the present investigations however, the tissues were fixed in 10% neutral buffered formalin for four hours at room temperature. This supports Adams (1957) view that prolonged fixation in formalin is not desirable.

Chakrabarti *et al.* (1994) in *Oreochromis mossambicus* reported complete absence of tryptophan in the intestine and rectum but weak reaction in the hepatic cells. However, the present histochemical investigations show
marked presence of tryptophan in the gills, intestinal bulb, intestine and the liver of the fish studied. The afferent epithelia, the chloride and pavement cells of the gills, mucosal border, columnar epithelial cells and submucosal blood vessels and blood capillaries of the intestinal bulb and intestine and the cytoplasm of the hepatic cells are the sites of localization. The occurrence of tryptophan in the said tissues of *Schizothorax curvifrons* is probably indicative of the presence of zymogen granules in them which have secretory role.

Elastic fibres are an important constituent of vertebrate connective tissue, particularly those with elastic properties such as arterial walls, lung alveoli, skin, tendon and ligaments. In the major blood vessels, 20-50% of the total protein is elastin. Elastic fibres from all sources are remarkably insoluble in inorganic or organic solvents, differing in this respect from collagen which dissolves, for instance in 2% acetic acid. This difference in reactivity may be connected with the fact that elastin contains 90% of non-polar amino acids and collagen contains only 50%.

Elastic fibres are very stable components, but with the advancing age they undergo marked changes taking the form of longitudinal splitting and breaking into fragments and ultimately into granules. These changes are associated with chemical changes in amino acid contents particularly raised levels of glutamic and aspartic acid and in calcium content up to 14%.

Although there are a large number of specific stains for localizing elastic fibres, the most important are those of Verhoeff's (Verhoeff, 1908) and Gomori's (cf. Pearse, 1972). In the present study however, Verhoeff's elastic stain (Verhoeff, 1908) has been adopted for the histochemical demonstration of elastic fibres.

It has been observed during the present study that the different portions of the intestinal tract react positively for elastic fibres. The elastic fibres are
mostly localized in the walls of the blood vessels and in the submucosal connective tissue. This is suggestive of the fact that elastomeric property of elastic fibres aid in the widening or distensibility of the intestinal tract to accommodate the food material, besides enhancing the rate of blood flow through the vessels carrying the absorbed food material. According to Pearse (1972), the elastic fibres owe their peculiar properties to the relative absence of basic amino acids in the molecule, wide spacing of the various histochemically reactive amino acid residues in the polypeptide chain and to the presence of oxidized phospholipids.

DNA is of utmost biological importance as it plays a vital role in cellular metabolism. In the present study, Feulgen reaction (Feulgen and Rossenbeck, 1924) has been employed for the histochemical localization of DNA. The Feulgen reaction is generally considered specific for DNA (Lillie, 1954). According to Pearse (1972), the Feulgen reaction can be applied after almost any fixative except Bouin's fixative with which the excessive hydrolysis occurs during fixation. However, during the present investigation, the tissues were fixed in Carnoy's fixative which has been considered as an excellent nuclear fixative by Lillie (1954). Kurnick (1955) considered 8-12 minutes hydrolysis optimum for obtaining the deepest stain. In the present study however, the hydrolysis time was maintained at 10 minutes in N-HCl at 60°C.

Gol'dshtein et al. (1952) and Deb and Banerjee (1957) observed that vitamin C regulates the viscosity and formation of DNA in the cells. In the present study however, the localization of DNA was carried out without providing any extra diet containing vitamin C.

The present histochemical observations reveal the presence of DNA in the tissues studied. The centrally placed nuclei of the chloride and pavement cells, the basal nuclei of mucous cells of the gills, nuclei of the columnar
epithelial cells, the scattered oval nuclei of the submucosal connective tissue and the centrally placed nuclei of the hepatic cells are observed to be sites of intense localization. The present observations and the earlier (Channa and Bhat, 2007 and Channa and Lone, 2007) reveal that intense reaction for DNA is indicative of large synthesis of proteins taking place in the tissues, suggesting high metabolic activity.

Glycogen commonly known as animal starch is a polysaccharide derived from and decomposing into glucose. The monomeric units (glucose) of glycogen are linked by glycosidic linkages in a highly branched molecule. Glycogen synthesis occurs virtually in all animal tissues but is especially prominent in the liver and skeletal muscles.

In the present investigation, Best's carmine method (Best, 1906) has been employed. The said method has been considered as the most satisfactory one by Pearse (1972). Srivastava (1966) histochemically demonstrated glycogen in the free border of absorptive cells of the intestine of three freshwater teleosts namely Mastacembelus panclus, Hetropneustes fossilis and Ophicephalus striatus. However, it has been observed during the present study that except for the liver which exhibits intensely positive reaction for glycogen in the cytoplasm of hepatocytes, the gills and the entire intestinal tract are devoid of glycogen. The present observations are in conformity with the findings of Chakrabarti et al. (1994); Abdelmeguid et al. (2002) and Channa and Lone (2002). The occurrence of glycogen in the hepatic cells probably indicates its role in various metabolic as well as physiological activities.

Mucous cell are of universal occurrence over the skin, on the gills and throughout the entire mucosal lining of the alimentary canal of teleosts. The mucous substances secreted differ both from species to species and also along the intestinal tract. The mucopolysaccharides are known to have a large
variety of functions, ranging from mechanical functions, through antimicrobial and antiviral to osmotic functions (Allen, 1981). The histochemical nature of mucous secreting cells has been the subject of a number of investigators (Moitra and Sinha 1972; Shafi, 1974; Fletcher et al., 1976; Gona, 1979; Reifel and Travill, 1979; Solanki and Benjamin, 1982; Sinha and Chakrabarti, 1984; Hidalgo et al., 1987; Srivastava et al., 1990; Prasad et al., 1992; Tibbets, 1997; Domenenghi et al., 1998; Diaz et al., 2001; Channa and Lone, 2002; Fiertak and Kilarski, 2002; Arellano et al., 2004; Diaz et al., 2005; Cinar and Senol, 2006 and Channa and Nabi, 2008).

Periodic acid Schiff's (McManus, 1946) and alcian blue (Mowry, 1956) techniques have been followed for the localization of neutral and acid mucopolysaccharides respectively in the tissue sections of the gills, intestinal bulb, intestine, rectum and liver of *Schizothorax curvifrons*. It has been observed that except for the liver which reacts negatively, the gills and the different portions of the intestinal tract reveal positive results for both neutral and acid mucosubstances. These observations are compatible with the studies made earlier (Sinha and Chakrabarti, 1984; Diaz et al., 2001, 2003; Arellano et al., 2004; Calabro et al., 2005; Petrinic et al., 2005; Cinar and Senol, 2006 and Channa et al., 2008a).

Mucous secreted by the mucous cells of the gill epithelia is involved in many biological activities such as swimming and defense against pathogenic microorganisms, parasites and pollutants (Fletcher, 1978). This secretion also has an important role in the ion regulation and diffusion of ions (Handy et al., 1989 and Diaz et al., 2001, 2005). The secretion of mucin by different mucous cells of the intestinal bulb and intestine keeps them moist and also provides lubrication to the food during passage thus enabling easy transport of the ingested material besides protecting the epithelial cells from mechanical injury (Sinha and Chakrabarti, 1982 and Pedini et al., 2001). The
predominance of mucin in the intestinal tract acts as a buffer in order to maintain optimum pH of the ingested food material for the action of various enzymes. Mucin also forms a favorable environment for ionic and molecular diffusion (Rosen and Comford, 1971). It is also presumed that occurrence of mucin may provide the co-factors required for enzymatic degradation of food (Domeneghini et al., 1999). On the other hand the presence of neutral and acid mucin in the rectal mucosa helps in easy defecation of undigested and underdigested food material.

Biological lipids are a chemically diverse group of compounds, the common and defining feature of which is their non-polarity and insolubility in water but show considerable solubility in non-polar organic solvents. The neutral or true lipids also referred as triglycerides are composed of three fatty acids each in ester linkage with a single glycerol.

Sudan black B method (McManus, 1946) has been employed for the localization of neutral lipids. Except for the gills, rest of the tissues studied during the present investigation reveal the presence of neutral lipids. The sites of intense localization include the brush border and the basal portions of the columnar epithelial cells of mucosa, submucosal lymph spaces and blood vessels including the capillaries and the hepatic cells. Intense stain intensity observed in the brush border and columnar epithelial cells of mucosa is indicative of the fact that these are the primary sites of absorption. This is in correlation with the findings of Al-Hussaini (1949); Sivadas (1965); Sastry and Garg (1978); Channa and Raina, (1983); Channa and Tiku (1983) and Denstadli et al. (2004). Pronounced reaction for lipids noted in the submucosal lymph spaces and blood vessels including the capillaries is probably due to the fact that these are the two pathways through which lipids are finally transported (Bykov, 1958 and Wilson, 1962).
Regarding the distribution pattern and stain intensity of lipids in the liver, the present observations are in accordance with the findings of Abdelmeguid et al. (2002), who have reported intense stain intensity throughout the cytoplasm of the hepatocytes of *Tilapia zilli*ii. Though the liver is not normally the chief accumulator of lipids, yet tends to keep its fat content relatively uniform even when large excesses of fat are being piled up in the adipose tissue thereby maintaining a nearly steady state of concentration of various lipids circulating in the blood. Lee et al. (1983) and Adams and Mclean (1985) are of the opinion that stored lipids are used to mediate the effects of stress and also serve as energy buffers during the periods of harsh environmental conditions and food shortages. According to Brauffaldi and Cucchi (1989), the presence of high lipid deposits may exert protective effects by removing and inactivating organic chemicals from the metabolism, thus improving toxicant tolerance and resistance.

Enzymes are the most remarkable and highly specialized proteins having extraordinary catalytic power, often far greater than that of synthetic or inorganic catalysts. They have a high degree of specificity for their substrates. A better knowledge of digestive enzyme activities is essential for a deeper understanding of the physiology of fish nutrition. As in other vertebrates, the ability of fish to utilize ingested nutrients depends on the presence of appropriate enzymes in appropriate locations of the wall and along the lumen of the intestinal tract.

The histochemical distribution and localization of alkaline phosphatase in the digestive system of various teleosts is well documented (Srivastava, 1966; Sivadas and Govindan, 1970; Goel and Sastry, 1973; Shaffi and Jafri; 1974; Goel, 1975; Sastry, 1975; Shafi, 1978; Sinha, 1979; Chakrabarti and Sinha, 1982; Kuz'mina and Smirnova, 1992 and Kozaric et al., 2004 and
The aforesaid workers have reported the presence of alkaline phosphatase activity in varying intensities at different locations. It has been observed during the present investigation, that the gills, intestinal bulb, intestine, rectum and liver depict the presence of alkaline phosphatase. Intense alkaline phosphatase activity noted in the gills indicate that the teleostean gills are the sites of several important physiological activities.

The absorption of digested food particles and molecules generally takes place along the brush borders and cytoplasm of enterocytes where numerous digestive enzymes are localized. The present observations and the earlier (Goel and Sastry, 1973; Shaffi and Jafri, 1974; Sastry, 1975; Shafi, 1978; Sinha, 1979; Chakrabarti and Sinha, 1982; Segner et al., 1989; Kuz'mina and Smirnova, 1992; Gawlicka et al. 1995; Tengjaroenkul et al., 2000 and Kozaric et al., 2006) reveal that the brush borders of enterocytes of mucosa, lamina propria and the submucosal lymph spaces, blood vessels and blood capillaries are the localized sites for alkaline phosphatase. According to Loyda et al. (1979), alkaline phosphatase is found primarily in the cell membranes where active transport takes place. Intestinal alkaline phosphatase is considered to be involved in the absorption of nutrients such as, glucose, lipids, calcium and inorganic phosphate (Cousin et al., 1987; Roubaty and Portmann, 1988; Harris, 1989; Dupuis et al., 1991, Mahmood et al., 1994 and Kozaric et al. 2006). The present findings undoubtedly suggest that digestion and absorption of metabolites occurs primarily in the intestinal bulb and intestine proper. The progressive decrease in alkaline phosphatase activity in the rectum of the fish studied possibly indicates that nutrient absorption is not an important function of this region (Kozaric et al., 2006). Intense alkaline phosphatase activity observed in the cytoplasm of hepatic cells is in accordance with the findings of Goel and Sastry (1973), Sastry (1975) and Chakrabarti and Sinha (1982). Alkaline phosphatase activity in the hepatic cells is mainly concerned with the formation of glycogen through
dephosphorylation process (Sinha et al., 1988). According to Srivastava (1966), alkaline phosphatase is firstly involved in the dephosphorylation process and so be concerned in the deposition of glycogen and secondly it possibly absorbs glucose.

Acid phosphatase is one of the marker enzymes for lysosomes but its activity has also been detected outside the lysosomes (Chi-Wei and Fishmann, 1972). It has been observed during the present histochemical study that acid phosphatase is present in the entire intestinal tract and liver.

Regarding the intensity and distribution pattern of acid phosphatase in the intestinal tract of Schizothorax curvifrons, the present observations are consistent with the findings of Rode and Frank (1967); Shaffi and Jafri (1974); Sastry (1975); Sinha (1979); Chakrabarti and Sinha (1982); Sinha et al. (1988) and Kozaric et al. (2004 and 2006), who have reported maximum activity for the enzyme in the brush borders of mucosa, columnar epithelial cells, the submucosal lymph spaces, blood vessels, blood capillaries and connective tissue of various species of fishes. Intense acid phosphatase activity noted in the mucosal epithelial cells and the submucosal layer can probably be correlated to their secretory and absorptive nature as also suggested by Sastry (1975) and Baglole et al. (1998). Acid phosphatase activity is related to pinocytotic activity and intracellular digestion in teleosts as well (Gauthier and Landis, 1972; Georgopoulou et al., 1986 and Govini et al., 1986). Well pronounced enzyme activity observed in the cytoplasm of hepatic cells is in conformity with the findings of Sastry (1975) and Chakrabarti and Sinha (1982).

Adenosine triphosphatase (ATPase) activity is observed to be present in the entire intestinal tract and liver of the fish studied. The present observations on the intensity and distribution pattern of the enzyme are in correlation with the findings of Sastry (1975 and 1976) who demonstrated
strong ATPase activity along the brush borders of mucosa, columnar epithelial cells and in the cytoplasm and nuclei of the hepatic cells of *Ophiocephalus punctatus* and *Heteropneustes fossilis* respectively. However, mild activity reported by him in the mucsularis and serosa of both the species of fishes studied has not been observed in the present study. Gawlicka *et al.* (1995) and Baglole *et al.* (1998) are of the opinion that small peptides and amino acids resulting from the action of digestive enzymes are transported with the collaboration of ATPase and alkaline phosphatase through intestinal enterocyte membrane. The present observations extend support to the views of the aforesaid workers. The adenosine triphosphatase (ATPase) also plays an important role in maintaining functional integrity of the cell membrane and other cellular activities.

Lipases are the enzymes of low specificity requiring only an ester linkage for their reaction. Several workers have histochemically reported the occurrence of lipase in the different parts of the digestive system of teleosts (Al-Hussaini, 1949; Sastry, 1974a, b; Goel, 1975; Swarup and Goel, 1975 and Tengjaroenkul, 2000).

The results of the present investigation demonstrate the presence of lipase in the intestinal bulb, intestine and liver of the fish investigated. According to Vonk (1937), lipase activity in the intestine is probably due to the adsorption of the pancreatic enzyme into the intestinal mucosa. The present observations however, clearly reveal that the intestinal mucosa is capable of secreting lipase as evidenced by intense activity noted in the brush border and cytoplasm of the columnar epithelial cells of both the intestinal bulb and intestine. The results further reveal that the lipolytic activity occurs primarily in the anterior two third portions of the intestine. These observations confirm the earlier findings (Sastry 1974a, b; Goel, 1975; Swarup and Goel 1975 and Tengjoraenkul *et al.*, 2000). The presence of lipase in the
submucosal lymph spaces, blood vessels and blood capillaries might be possibly due to the hydrolysis of fats during transport in these vessels. The occurrence of lipase in the intestinal bulb and intestine and its complete absence from the rectum may probably be related to the low fat content in plant material naturally consumed by *Schizothorax curvifrons*.

There is no conclusive evidence about the secretion of lipase by liver, though its presence has been reported in the combined liver and pancreas (Ishida, 1936 and Sarbahi, 1951). However, the present observations showing intense lipase activity in the cytoplasm around the nucleus of the hepatic cells of the fish studied. These observations are not only consistent with the findings of Sastry (1974a, b) but also extend support to the view of Barrington (1957) who states that the secretion of lipase might be a property of hepatic tissue itself.

5.2. ULTRASTRUCTURE

5.2.1. Scanning Electron Microscopy (SEM)

Scanning electron microscopic observations on the surface ultrastructure of the gills of *Schizothorax curvifrons* reveal the presence of well developed and compactly organized primary filaments which radiate from the gill arches. Both sides of the primary lamellae are noted to be lined up by secondary lamellae as also reported earlier in other teleosts (Kudo and Kimura, 1984; Ojha *et al.*, 1987 and Arellano *et al.*, 2004). The gill filaments and their secondary lamellae represent two general types of epithelium (Laurent and Dunel-Erb, 1980; and Laurent, 1984).

Filaments or the primary lamellae constitute the most prominent respiratory structures of the gills. Laurent (1984) states that the shape of the gill filaments in general varies from being very filamental to fairly stubby
structures. However, only filamental type of gill filaments are observed in the present study though the length exceeds the breadth. According to Hughes (1966), the number of filaments do not increase so markedly in adult fishes as during the juvenile growth period, although each filament exhibits significant increase in the length as the fish grows. This leads to an increase in the total length of all the filaments, which is an important morphometric dimension used in calculating the gill area. In *Schizothorax curvifrons* the gill filaments observed are long and narrow projections lateral to the gill arch that taper at their distal ends. The secondary lamellae are found evenly distributed along the length of the filament and are perpendicular to the long axis of the filament besides being parallel to each other. This is in conformity with the findings of Kudo and Kimura (1984) and Evans *et al.* (2005). The lamellae constitute the most important units of the gill system from the point of view of gas exchange (Laurent, 1984). According to Evans *et al.* (2005), the lamellae not only dramatically increase the surface area of the gill filament epithelium and result in small diffusion distance between the blood that perfuses each lamellae and the respiratory water but are also well suited for diffusive loses or gains of ions and water to or from the environment. From a design point of view, lamellae are required to have a large surface area where gas exchange can be facilitated without any excessive exchange of ions and water. A close contact between water and blood must be achieved so that oxygen uptake can occur in a limited period during which the water and blood are passing the lamellae.

Ojha *et al.* (1987) observed two types of gill rakers in the gills of freshwater mullet, *Rhinomugil corsula* and single type of gill rakers in *Sicamugil cascasia*. However, during the present study, only single type of gill rakers are noticeable. The gill rakers are long and slender bearing minute projections on the inner side. These projections were also observed by Ojha *et al.* (1987) on the first type of gill rakers of the gills of *Rhinomugil corsula*. 
The well developed gill rakers and their specific orientation may possibly be related to the herbivorous nature of the fish studied. The densely packed rakers aid in the detection and capturing of food flowing through water. The presence of microridges on the gill rakers can be associated with the holding of mucous secreted by the goblet cells. The mucous serve to increase the efficiency of gill rakers by cleaning the surface and exposing the chemoreceptors for the detection of food and chemical characteristics of ambient water. Moreover, the arrangement and density of gill rakers are specific for different fishes and may be a guiding factor for determining food and feeding habits and the taxonomic status of the fish.

The filament epithelium of teleosts consists of pavement cells, mucous cells and chloride cells (Morgan and Tovell, 1973; Kendall and Dale, 1979; and Fernandes and Perna-Martins, 2001) as is also evident from the present investigation. The major surface area of the gill filament is covered by pavement cells, which are largely considered to play a passive role in gill physiology and are significant for gaseous exchange. The microridges are noted to be present in between the pavement cells defining their cell boundaries. The presence of microridges on the apical membrane of pavement cells seem to increase the functional surface area of the epithelium and may also play a role in anchoring mucous to the surface (Hughes and Wright, 1970; Lewis, 1979; Fernandes and Perna-Martins, 2001 and Evans et al., 2005). According to Olson and Fromm (1973), the nature of the microridges varies in fish species but no correlation between microridge structure and the physiology and ecology has yet been established.

The mucous cells are more or less similar to those found in the gills of other teleosts (Laurent, 1984 and Fernandes and Perna-Martins, 2001). The mucous secreted by the mucous cells have different roles such as control of infection, prevention of dehydration, ion regulation and ion diffusion (Handy
et al., 1989 and Diaz et al., 2001). In addition, the mucous of the gill epithelia is also involved in swimming and defense against pollutants and parasites (Fletcher, 1978). Schizithorax curvifrons is a freshwater fish living generally in ion poor waters. The released mucin plays a significant role in the prevention of loss of ions.

The chloride cells in the gills of the fish studied are found close to the onset of secondary lamellae. Similar observations have been made by Fernandes and Perna-Martins (2001) with respect to the gills of Hypostomus plecostomus. Franklin and Davison (1989), while working on the scanning electron microscopy of the gills of freshwater adopted sockeye salmon, Oncorhynchus nerka, have reported two morphologically different chloride cells on the afferent surface of the gill filament. However, during the present study, only single type of chloride cells are noted to be present on the gill epithelium. Teleostean chloride cells seem to play an important role in ionic and osmotic regulation (Kamaky, 1986). According to Evans et al. (2005), chloride cells occupy a much smaller fraction of the branchial epithelia, but they are considered to be the primary active sites of physiological processes in the gills.

Scanning electron microscopic studies of the intestinal bulb and intestine reveal that the mucosa is thrown in to zigzag pattern of folds. However, the mucosal folds of the intestinal bulb are observed to be much larger and more complex than that of the intestine. Similar observations have been made earlier in other teleosts (Vickers. 1962; Sinha, 1983 and Caceci. 1984). Al-Hussaini (1949) in Cyprinus carpio reported that the mucosal folds of the intestinal bulb are thrown in various directions, branch and reunite and the direction of the caudal intestine is predominantly transverse but some zigzagging is present. However, no such branching and reunion has been observed in the intestinal bulb of the fish studied. The present observations
further reveal that a distinct concavity is noticeable in between the folds of intestinal bulb and intestine. The rectal mucosa on the other hand is thrown in to irregularly arranged mucosal folds. The minor folds are totally absent. The mucosal folds are covered with a distinct film of mucin and the pores through which the mucous cells expel their contents are also quite distinct.

The presence of major mucosal folds along with the minor ones aid in increasing the mucosal surface of the intestinal bulb and intestine, whereas the distinct cavities in between the mucosal folds serve in the retention of ingested food for longer periods, so essential for the effective functioning of the intestine (Sinha, 1983 and Grau et al., 1992).

The occurrence of major mucosal folds and the absence of minor mucosal folds as well as well defined cavities in the rectal mucosa are suggestive of its insignificant role in food storage, digestion and absorption. However, a distinct film of mucin observed in the rectal mucosa indicates its role as a lubricant helping thereby in easy defecation of the undigested or underdigested food. The secretion of mucin to the exterior through pores has been reported by various workers in different teleosts (Kapoor, 1958; Khanna, 1964, 1968; Moitra and Sinha, 1971, 1972; Sinha and Moitra, 1975, 1976 and Moitra and Ray, 1977, 1979).

Sinha (1983) in adult major carp, Labeo rohita and Sinha and Chakrabarti (1985) in Calla calla have reported the presence of microridges on the luminal plasma membrane of the columnar epithelial cells of the intestinal tract which is also evident from the present study. Microridges are not only restricted to the intestinal segment but have also been observed in the fish skin (Hawkes, 1974; Lanzing and Hugginbotham, 1974; Harris and Hunt, 1975; Hunter and Nayudu, 1978 and Bereiter-Hahn et al., 1979), in the epithelial cells of gills (Rajbanshi, 1977; Olson and Fromm, 1973 and Mattey et al., 1979) and in the stratified epithelial cells of buccopharynx and
oesophagus (Mallatt, 1979; Sis et al., 1979 and Ezeasor and Stokoe, 1980). Therefore the occurrence of microridges on a variety of teleost epithelial cells probably indicates some common functions. Sinha (1983) reported that microridges represent a mechanical adaptation which in the anterior intestine of adult *Labeo rohita* would withstand the trauma resulting from ingested materials. Sperry and Wasersug (1976) also reported similar functions of microridges in the oesophageal region of trout. In addition, these microridges also help in holding and spreading a thin film of mucin secreted by the adjacent mucous cells.

5.2.2. Transmission Electron Microscopy (TEM)

Regardless of the lineage, majority of the fishes use the gills as the primary site of aquatic respiration. Gills are the main sites for molecular exchange between the internal environment of fish and its external media (Olson, 1996) such as gas transfer, acid base regulation (Randall et al., 1982) and ionic regulation (Eddy, 1982).

Transmission electron microscopic studies of the gills of *Schizothorax curvifrons* reveal that the filament and the lamellar epithelia are equipped with the cells similar to those described for other teleosts (Morgan and Tovell, 1973; Laurent, 1984; Fernandes and Perna-Martins, 2001; Arellano et al., 2004 and Diaz et al., 2005).

Dunel-Erb et al. (1982) identified neuroepithelial cells on the filament epithelium of *Oncorhynchus mykiss, Stizostedion lucioperca, Ictalurus melas, Anguilla anguilla* and *Micropetes dolomieu*. However, such cells are not found on the filament epithelium of the fish studied. This may possibly be due to the particular location of these cells on the epithelium or due to the orientation of the sections.

The distribution and number of mucous cells in gill epithelia may vary from species to species (Laurent, 1984). However, regarding the distribution
and number of mucous cells, the present observations are in correlation with the findings of Fernandes and Pema Martins (2001) and Diaz et al. (2005) who observed mucous cells on the primary and secondary gill lamellae of *Hypostomus plecostomus* and *Micropogonias furnieri* respectively. Mucous cells are noted to be occupied by mucous globules of varying electrodensities due to which their nuclei are displaced towards the basal region of the cells. Variations in the electrodensity of mucous globules are either due to their degree of development or due to their different chemical composition.

Perera (1993) could not observe the presence of desmosomes between the mucous cells and adjacent epithelial cells in the gills of *Scomber australasicus*. In the present study also no clue or mark of identification of desmosomes is noticeable although cellular interdigitations are distinctly noted between the mucous cells and their neighbouring cells. The presence of cellular interdigitations between the mucous cells and their neighbouring cells in the gills of *Micropogonias furnieri* has been reported by Diaz et al. (2001).

Mucous cells of teleosts may secrete mucous either by the fusion of vesicles and the expulsion of an amorphous mass (Mittal et al., 1980 and Laurent, 1984) or by the secretion of whole vesicles as observed by Harris and Hunt (1975) and also in the present study. The mucous cells secrete mucous forming a protective layer besides facilitating ion regulation.

Pavement cells located on the primary lamellae of the gills of the fish studied bear microridges on their apical membrane which are covered with glycocalyx. However, microridges are not found on the apical membrane of the pavement cells of the secondary lamellae. The absence of microridges on the surface of secondary lamellae has been reported in a number of teleosts (Moron and Fernandes, 1996; Arellano et al., 2004 and Diaz et al., 2005). Nevertheless, Fernandes and Pema-Martins (2001) reported the presence of microridges on the pavement cells of secondary lamellae of *Hypostomus*
Plecostomus. Microridges have been proposed to prevent the loss of mucous from the epithelial surface besides providing structural integrity to the membrane (Olson and Fromm, 1973; Sperry and Wassersug, 1976 and Diaz et al., 2005). The glycocalyx that lines the microridges may also contribute to the retention of mucous by reducing the abrasive action of molecules suspended in water (Carmona et al., 2004). The occurrence of smooth pavement cells without microridges in the secondary lamellae has been related to functional differences between both lamellae. Kultz et al. (1995) speculated that the absence of microridges on the pavement cells of secondary lamellae could facilitate gas exchange by minimizing the mucous layer on the epithelial surface and by enhancing the water flow through the secondary lamellae. Evidences suggest that the pavement cells of freshwater teleost gills may play an active role in ion uptake and acid base regulation (Goss et al., 1992; Perry et al., 1992; Perry and Laurent, 1993 and Evans et al., 2005).

Another noticeable feature of the gill epithelia of the fish studied is the presence of chloride cells which are observed to be long and round with spherical nucleus, great number of mitochondria and a well developed intercytoplasmic membrane system. Similar observations have been made by Fernandes et al. (1998) and Diaz et al. (2005) with respect to the gills of Hypostomus plecostomus and Micropogonias furnieri respectively. Two types of chloride cells referred to as α and β chloride cells have been distinguished in different species of teleosts. However, only one type of chloride cells are discernible in the gills of the fish studied.

Laurent (1984 and 1989) reported that the distribution of chloride cells is normally restricted to the interlamellar region of the filament epithelium at the base of the lamellae, being more abundant on the trailing edge of the filament. In the present study, however, the chloride cells are found not only throughout the lamellae but also on the leading and trailing edges of the
filament. Fernandes and Perna-Martins (2001) also reported similar findings in the gills of *Hypostomus plecostomus*. The presence of chloride cells in the lamellar epithelium of freshwater fishes varies according to the species; being absent in guppy (Pisam *et al*., 1987) while frequently observed in goldfish (Kikuschi, 1977). Perry and Laurent (1989) established a correlation between a number of chloride cells and Ca\(^{2+}\) or NaCl concentration in water and gill ion uptake. They further suggested that these may be the sites for such ion transport. The frequent proliferation of chloride cells on the lamellar epithelium is shown by the fish living in ion poor water while a fish living in high ion concentration do not show chloride cells on the lamellar epithelium (Mattheij and Stroband, 1971; Laurent and Hebibi, 1989 and Perry and Laurent, 1993). Therefore the presence of chloride cells on the lamellar epithelium of *Schizothorax curvifrons* may be an adaptation to the poor ion concentration in the water which may be beneficial to enhance the ion transport capacity of the gill.

It has been suggested that the large size of the chloride cells increases the water blood barrier and also affect the transference of respiratory gases thereby decreasing resistance to a hypoxic environment (Thomas *et al*., 1988; Bindon *et al*., 1994; Greco *et al*., 1995 and Fernandes *et al*., 1998). In general the chloride cells of freshwater teleosts share some ultrastructural characteristics with those from seawater teleosts (Perry *et al*., 1992; Laurent and Perry, 1995 and Perry, 1997). However, important differences such as the accessory cells and multicellular complexes associated with chloride cells in seawater teleosts are not common in freshwater teleosts. The chloride cells are the active sites of chloride secretion and are also related to pH, salinity and low concentration of Ca\(^{2+}\) in water (Laurent *et al*., 1985; Leino *et al*., 1987 and Diaz *et al*., 2005).
The accessory and the rodlet cells are not found in the gill epithelia of *Schizothorax curvifrons*. According to Laurent and Dunel-Erb (1980), a transferred fish from seawater to freshwater is accompanied by total disappearance of accessory cells, although this process can be reversible. This confirms that the gills of fresh water teleosts are almost devoid of accessory cells.

General fine structures and the organization of enterocytes of the entire intestinal tract the fish studied are more or less similar to those of other teleosts (MacDonald, 1987 and Arellano et al., 2002). Kuperman and Kuz’mina (1994) observed an increase in the height of microvilli from the anterior portion to the posterior portion of the intestine of bream, *Abramis brama*. However, it has been noted during the present study that the height of the microvilli show a progressive decrease from the intestinal bulb to the rectum. The microvilli forming the brush border increase the digestive and transport surface of the intestinal tract. Within the microvilli of the intestinal bulb and the intestine, many microfibrills project into the apical part of the cytoplasm where a terminal net is seen. Below the terminal net, numerous invaginations of the apical membrane are observed. The cytoplasm of the enterocytes contains rough endoplasmic reticulum near the nucleus, whereas the smooth endoplasmic reticulum is located along the basal portions. Besides the assemblage of mitochondria whose profile varies from spherical to elongate are discernible in the supra nuclear region of the enterocytes throughout the intestinal tract. These observations are consistent with the findings made earlier (Yamotto, 1966; Kayanja et al., 1975; Stroband, 1977, Noaillac-Depeyre and Gas, 1979; Anderson, 1986; MacDonald, 1987; Olsen et al., 1999 and Gallagher et al., 2001).

The present observations further reveal the presence of lipid droplets of different sizes in the cytoplasm of the enterocytes throughout the intestinal
tract. The occurrence of lipids in the cytoplasm of enterocytes suggests that the fish stores excess of lipids during digestion and absorption (Sire et al., 1981; Kuperman and Kuz’mina, 1994; Murray et al., 1996 and Arellano et al., 2002).

Abaurrea-Equisoain and Ostos-Garrido (1996) observed two morphologically different types of enterocytes in the intestinal epithelium of rainbow trout, *Oncorhynchus mykiss*. According to them, one type of enterocytes is characterized by electrodense cytoplasm while the other type has an electron clear cytoplasm. However, only one type of enterocytes having an electron clear cytoplasm has been observed in the intestinal tract of fishes (Kuperman and Kuz’mina, 1994 and Arellano et al., 2002) and also in the present study. The enterocytes are noted to be interconnected in the apical region by desmosomes.

A notable feature observed only on the intestinal microvilli of *Schizothorax curvifrons* is the presence of spherical blebs. The presence of blebs on the intestinal microvilli of the fish studied is indicative of its role in increasing the apical surface of the enterocytes as also suggested by Kuperman and Kuz’mina (1994). According to Eletski and Tsibulevski (1979), the effect of penetration of molecules of water through the hydrophobic region of the lipid phase of the membrane becomes possible in parts of a curved membrane when the radius of curvature is 40-80nm and less and is usually observed on the tips and at the base of the microvilli. The blebs probably have the same role (Kuperman and Kuz’mina, 1994).

Another ultrastructural characteristic noted in the entire intestinal tract is the presence of mucous cells. The mucous cells are occupied by high density secretory granules, pushing their nuclei towards the basal regions. The mucous cells secrete mucous which perform a number of functions such as lubrication, defense against pathogenic microorganisms, aid in the absorption
and transport of macromolecules besides acting as cofactors for various enzymes (Kuperman and Kuz'mina, 1994; Radaelli et al., 2000 and Arellano et al., 2002). The mucous also acts as a physiological barrier between the mucosa and environmental agents (Pajak and Danguy, 1993).

The occurrence of rodlet cells in the digestive tract of teleosts has been reported by several workers (Dezfuli et al., 1998; Arellano et al., 1999 and 2002). However, Murray et al. (1996) reported the total absence of rodlet cells from the digestive system of winter and yellow tail flounder. It has been observed during the present investigation that a number of rodlet cells are present in the epithelium of intestine, although the intestinal bulb and the rectum are noted to be totally devoid of rodlet cells. The function of the rodlet cells has not been fully understood as yet though their role in secretion and enzymatic functions has been suggested (Leino, 1982).

Gonzalez et al. (1993) and Brusle and Anadon (1996) have reported that the hepatocytes of the fish liver are relatively poor in organelles, indicating a low synthetic activity for secretory proteins. The present investigation on the TEM studies of the liver show the presence of centrally placed nucleus with two prominent nucleoli and distinct nuclear pores in the double nuclear membrane. Abundance of rough endoplasmic reticulum with parallel stacks of cisternae are noted to be located around the nucleus and along the plasma membrane. The cisternae of the rough endoplasmic reticulum are found to be filled with homogenous deposits of low density material which are uniform attachment of ribosomes with no free polysomes. The mitochondria varying in shape from circular to elongated are observed to be in close association with rough endoplasmic reticulum. These ultrastructural observations are compatible with the studies made earlier (Kendall and Hawkins, 1975; Hinton and Pool, 1976; Hugla and Thome, 1999; Yang and Chen; 2003; Vicentini et al., 2005 and Ribeiro et al., 2006).
Chapman (1981) reported the absence of golgi apparatus and smooth endoplasmic reticulum in the hepatocytes of rainbow trout, *Salmo gairdneri*. In the present study also, the said organelles are noted to be absent from the hepatocytes of *Schizothorax curvifrons*.

The cytoplasm of the hepatocytes of the fish studied show the presence of high content of scattered glycogen. Hinton and Pool (1976) and Vicentini *et al.* (2005) also noted fairly good content of glycogen in the cytoplasm of hepatocytes of *Oreochromis niloticus* and *Ictalurus punctatus* respectively. The presence of glycogen in the cytoplasm of hepatocytes is a characteristic feature of various fishes (Hampton *et al.*, 1985; Moon *et al.*, 1985; Gonzalez *et al.*, 1993).

Many investigators have reported the occurrence of intrahepatic exocrine pancreatic cells in teleosts and have suggested a secretory role to these cells (Kendall and Hawkins, 1975; Hinton and Pool, 1976; Marconi Stipp *et al.*, 1980; Eurell and Haensly, 1982; Beccaria *et al.*, 1992 and Vicentini *et al.*, 2005). Such cells have also been observed in the present study. These cells are clearly distinguishable from the hepatocytes by the presence of secretory granules delimited by a single membrane being uniform in density.