5.1. Histochemistry

The ubiquitous distribution and physiological significance of proteins make them an integral part of every biological activity. Proteins have high degree of specificity. In the present study, Mercury Bromophenol Blue method (cf. Pearse, 1972) has been employed for the histochemical localization of proteins. Pearse (1972) considers the said method to be most specific and a convenient one for histochemical demonstration of general proteins.

It has been observed in the present investigation that the stratified epithelial cells of the buccopharynx and oesophagus of *Schizothorax esocinus* exhibit intense stain intensity. Regarding the intensity and distribution of proteins in the intestinal tract, the present observations are in accordance with the findings of Medeiros et al. (1970), who has reported intense reaction for proteins in the intestinal tract of *Pimelodus maculatus*. The presence of high concentration of proteins in the columnar epithelial cells of the entire intestinal tract is probably indicative of the fact that the intestinal tract has the ability to absorb proteins, as has also been suggested by Stromband and Van der Veen (1981).

In the acinar cells of intrahepatic pancreatic tissue, the reaction is observed to be more pronounced than the hepatocytes, confirming greater amount of proteins in the pancreatic acini. The occurrence of high protein content in the intrahepatic pancreatic tissue is due to abundance of zymogen content (Gisbert et al., 1999). It may be concluded that the high concentration of proteins in the tissues studied is indicative of its role in synthetic activities.

Tyrosine is one of the twenty standard amino acids that is used by cells to synthesize proteins. The said amino acid is an aromatic amino acid and is derived from phenylalanine by hydroxylation in the para position.
Millon reaction modified by Bensley and Gersh (cf. Pearse, 1972) has been adopted during the present investigation for the histochemical demonstration of tyrosine. According to Gibbs (1927) the reaction proceeds in two stages: (i) a nitrosophenol is produced by the substitution of NO for H ortho or meta to the hydroxyl of the phenol and (ii) Hg$^{2+}$ is incorporated into a new ring by chelation which includes the nitrogen of the nitroso group. The new complex thus formed is red in colour. Ostaszewska (2002) reported the presence of zymogen granules containing tyrosine in the pancreatic cells of Aspius aspius. The results of the present investigation reveal the presence of tyrosine in all the tissues of digestive system of the fish studied. The stratified epithelial cells, columnar epithelial cells of the mucosa, the submucosal blood capillaries and connective tissues, the muscularis and the intrahepatic pancreatic acini are observed to be the sites of localization. The tissue proteins usually contain tyrosine as one of the constituent amino acids. This may perhaps be the reason for the occurrence of tyrosine in all the tissues examined in the present study.

Tryptophan is one of the essential aromatic amino acids and belongs to the neutral amino acid group. Although specific histochemical reactions are available for the localization of tryptophan (Adams, 1957; Glenner and Lillie, 1957 and Bruemmer and Thomas, 1958), the p-Dimethylaminobenzaldehyde (DMAB)-Nitrite method (Adams, 1957) has been employed for the localization of tryptophan in the present study. Pearse (1972) suggests that the specificity of DMAB-nitrite reaction is high. According to the same author, the said method can be conveniently used in the laboratory for histochemical findings. Adams (1957) prefers alcoholic fixatives to those containing formaldehyde. However, in the present investigation, the tissues were fixed in 10% neutral buffered formalin though the time of fixation was limited to four hours. This extents support to the views of Adams (1957) that longer fixation period in formalin is not desirable.
Adams (1957) observed the intense reaction for tryptophan in tissue components like the fibrin, fibrinoid, paneth cell granules, chief cell granules, zymogen granules, thyriod, colloid, muscle fibres, neurokeratin and inner hair-root sheath and moderate reaction in liver parenchymal cells, keratin and the deeper layers of the epidermis, the cytoplasm of many neurons and the cytoplasm of the cells of the peripheral parts of argentaffin carcinomas.

Chakrabarti et al. (1994) histochemically observed entire absence of tryptophan from the buccopharynx, oesophagus, intestine and rectum of *Oreochromis mossambicus*. The results of the present investigation, however, reveal positive reaction for tryptophan in the entire intestinal tract and rectum. The mucosal epithelial cells, the submucosal blood capillaries and connective tissue, muscularis and the serosa are sites of localization. Regarding the distribution of tryptophan in the hepatopancreas, the present observations are in agreement with the findings of Chakrabarti et al. (1994) who has observed intense stain intensity in the intrahepatic pancreatic acinar cells than the hepatic cells of *Oreochromis mossambicus*, confirming thus the presence of high amount of tryptophan. The occurrence of tryptophan in the said tissues of *Schizothorax esocinus*, is probably indicative of the presence of zymogens in them, which have secretory role.

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 elastic contains 90% of non-polar amino acids and collagen only 50% (Pearse, 1972). Elastic fibres are composed of two components: the amorphous core of elastin and microfibrils, of which fibrillin is the primary element (Hadley-Miller et al., 1994). Elastic fibres are very stable component of the connective tissues and vessels.
However, marked changes occur, with advancing age, as they take the form of longitudinal splitting, breaking into fragments and ultimately into granules. These changes are associated with chemical changes in amino acid content and in calcium content.

Elastic fibres are distinguishable by means of a number of “specific” stains, the most important of these being those of Verhoeff (1908) and Gomori (1950). Horobin and James (1970) stained elastic fibres with Direct Blue 152 and described the general hypothesis for the staining of elastic fibres. Yu and Lai (1970) described the structure of aortic elastic fibre electron microscopically by staining with Ruthenium Red. However during the present investigation, Verhoeff’s elastic stain (Verhoeff, 1908) has been used for the histochemical localization of elastic fibres.

It has been observed during the present study that except the intrahepatic pancreatic tissue, rest of the tissues reacts positively for elastic fibres. The distribution of elastic fibres is observed to be located around the walls of blood vessels in the dermis, submucosa, muscularis and serosa. This is indicative of the fact that the elastic fibres exhibit the properties of an elastomer that help in the distensibility of the digestive tract to accommodate the food material. Moreover, due to their power of distensibility, the blood flows quickly and without any hindrance, carrying the absorbed food material.

Feulgen reaction (Feulgen and Rossenbeck, 1924) has been employed for the histochemical detection of DNA in the histological sections of the digestive system of *Schizothorax esocinus*. According to Lillie (1954), the Feulgen reaction is generally considered specific for DNA. Pearse (1972) states that the Feulgen reaction can be applied after almost any fixative except Bouin’s fixative with which excessive hydrolysis occurs during fixation. However, during the present study, the tissues were fixed in Carnoy’s fixative which is considered as an excellent nuclear fixative by Lillie (1954). Kurnick
(1955) considered 8 to 12 minutes hydrolysis as 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.

According to Gol’dshtein et al. (1952) and Deb and Banerjee (1957), vitamin C regulates the viscosity and formation of DNA in the cell nucleus and is useful for its localization. However, during the present study, localization of DNA was carried out without providing any extra diet to the fish containing vitamin C.

The present histochemical investigations reveal the presence of DNA in the entire digestive system of the fish studied. Centrally placed nuclei of the stratified epithelial cells, the hepatic cells, the pancreatic acinar cells and the basal nuclei of the columnar epithelial cells of the mucosa are observed to be the sites of intense localization. The intense reaction for DNA observed in the tissues studied is indicative of its role in the large synthesis of proteins taking place in these tissues. There observations are compatible with the studies made earlier (Channa and Bhat, 2007; Channa and Lone, 2007 and Channa et al., 2008).

Glycogen is the main carbohydrate reserve store in animal tissues and composed of chains of D-glucose units, linked $\alpha$-1,4 except at points, of branching where the linkage is $\alpha$-1, 6 and are readily hydrolysed to glucose by boiling with dilute acids (Pearse, 1972).

Best’s Carmine method (Best, 1906), considered to be the most satisfactory one by Pearse (1972), has been adopted for the histochemical localization of glycogen in the present investigation. Pearse (1972) opined that the preservation of glycogen not only varied from one fixative to another but also with the duration of fixation. However, in the present study, absolute ethanol was used as a fixative for glycogen and the fixation time was maintained at 2-4 hours at room temperature. The overall superiority of
Discussion

absolute ethanol in the fixation of glycogen has been reported by Trott (1961) and Kugler (1965). Lillie (1947) furnished a detailed account on the preservation and histologic demonstration of glycogen. Sacks et al. (1957) reported the intracellular distribution of liver glycogen. Srivastava (1966) demonstrated histochemically the presence of glycogen in the mucosa of the intestinal tract of Mastacembelus panculus, Heteropneustes fossilis and Ophicephalus striatus.

It has been observed during the present histochemical investigation, that except for the liver which shows positive reaction for glycogen in the hepatic cells, rest of the parts of digestive system studied are observed to be devoid of any reaction for glycogen. Regarding the distribution of glycogen in the liver, the present observations are in agreement with the findings of Chakrabarti et al. (1994), Channa and Lone (2002), Sakr and Jamal Al Ilail (2005) and Sheibani and Yali (2006) who have demonstrated glycogen in the cytoplasm of the hepatic cells of various species of fishes. The presence of glycogen in the hepatic cells is probably indicative of its role in various metabolic as well as physiological activities.

The mucosubstances occur in the tissues as mixture of often very different individual components and they form a lubricating solution with water called mucous. Mucous cells of the alimentary tract in fishes are the main source of mucin. Their distribution and numerical abundance vary in different teleosts according to the type of food which they normally ingest (Khanna, 1968 and Sinha and Moitra, 1975).

The histochemical nature of mucous secreting cells has been extensively studied by various authors in different species of fishes (Khanna and Mehrotra, 1970; Gona, 1979; Shafi, 1979; Sinha and Chakrabarti, 1982; Prasad et al., 1992, Ostos Garrido et al., 1993, Chakrabarti et al., 1994; Nachi et al., 1998; Morrison and Wright, 1999; Channa and Lone, 2002; Domeneghini et al.,
The mucous cells in the digestive tract of fishes contain different types of mucosubstances, either acidic or neutral or a combination of both (Sinha and Chakrabarti 1982).

The histochemical detection of neutral and acid mucin in the digestive system of *Schizothorax esocinus* has been studied by employing PAS (Mc Manus, 1946) and AB (Mowry, 1956) techniques respectively. It has been observed during the present study that the oesophagus, intestinal bulb, intestine and rectum stain positively for both neutral and acid mucin. The buccopharynx on the other hand, depicts only the presence of neutral mucin, whereas, the hepatopancreas is negative to both PAS and AB tests.

The peripheral secretory and deeper non-secretory mucous cells in the buccopharynx react intensely for neutral mucin. Profuse secretion by the mucous cells of the buccopharynx keeps the region moist for smooth passage of food (Chakrabarti et al., 1994).

Intensely positive PAS and AB reactions observed in the oesophageal mucous cells is in accordance with the findings of Reifel and Travill (1977), Sinha et al. (1988), Chakrabarti et al. (1994), Petrinec et al. (2005) and Diaz et al. (2006). The secretion of mucin in this region is essential for food lubrication and also minimizes the mechanical abrasion during the passage of food through this region (Chakrabarti et al., 1994).

The secretion of mucin by different mucous cells of the intestinal bulb and intestine of the fish studied is suggestive of its active participation in the lubrication of food material for onward progression into the rectum. A possible role of intestinal mucin in osmoregulation has also been suggested (Narasimham and Parvatheswarao, 1974).
The studies of Ribelles et al. (1995) have shown that the quality of gut mucosubstances is directly related to environmental conditions, which in turn may directly affect the functions of the alimentary tract. The occurrence of mucosubstances in the intestine, possibly regulate the transfer of proteins or a fragment of them, as well as of ions and fluids (Stromband and Van Der Veen, 1981; Gupta, 1989; Sire and Vernier, 1992; Segner et al., 1994; Domeneghini et al., 1998 and Arellano et al., 1999).

The presence of neutral and acid mucin in the rectal mucosa helps in easy defaecation of undigested or underdigested food material. This investigation as well as the previous studies in other species of fishes (Chakrabarti, et al., 1994) point to the fact that the gut mucosubstances sustain functional harmony of the digestive tract.

Lipids are the heterogeneous group of substances that are generally soluble in organic solvents but insoluble in water.

Wolman (1950) used PAS reaction for the demonstration of lipids. However, in the present investigation, Sudan black B method has been employed for the histochemical localization of neutral lipids (Mc Manus, 1946). The results of present histochemical study reveal the presence of neutral lipids in the oesophagus, intestinal bulb, intestine, rectum and liver of the fish studied. The localized sites include the mucosal epithelial cells, the submucosal lymph spaces, blood vessels, blood capillaries, the muscularis and the serosal layer. Intense stain intensity observed in the mucosal epithelial cells, is in accordance 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). The presence of lipids observed in the submucosal lymph spaces and blood vessels is probably due to the fact that these are two path ways through which lipids are finally transported (Bykov, 1958 and Wilson, 1962). Gohar and Latif (1963) reported the occurrence of lipids in the muscle layers of
gastrointestinal tract of *Clarias lazera*, which has been confirmed by the present histochemical study. The serosal layer also stained positively for lipids.

The occurrence of neutral lipids in the hepatocytes of teleosts has been reported by Channa and Tiku (1983) and Abdelmejuid *et al.* (2002). Abdel-Aziz and El-Nady (1993) are of the view that the liver stores the major part of lipids. The present observations extend support to the findings of aforesaid workers in that the intense stain intensity for neutral lipids is observed in the cytoplasm of the hepatic cells.

Iron occurs in tissues mostly in the ferric state (Gomori, 1936). Perl’s Prussian blue method (*cf.* Pearse, 1972) has been employed in the present study for the histochemical demonstration of iron. The said method is considered to be specific for the detection of iron (Pearse, 1972). The present study reveals the presence of iron only in the liver. Intense reaction observed in the hepatocytes suggests that the iron is stored in some quantity in the liver and is utilized for various physiological needs, as has been also reported by Mitchell (1956). Total absence of iron observed in rest of the tissues of the digestive system studied may be due to the fact that either the amount of iron in the natural diet is too small to be detected histochemically or even if absorbed, it is immediately transported to the circulatory system for various metabolic needs.

Although, a few methods are known for the detection of calcium in the tissues (Cameron, 1930; Carr *et al.*, 1961 and Mc Gee-Russel, 1958). Dahl’s (1952) Alizarin red S method is considered to be reliable as it chiefly stains calcium deposits (Pearse, 1972). According to the same author, the method of Dahl (1952) is more sensitive as compared to other methods. Even Dahl (1952) regards the sensitivity of the Alizarin red S method “to be greater when used histochemically”. Besides, the said method can be conveniently applied at room temperature. No adhesive was used for mounting the sections in the present study because it has been observed by Dahl (1952) that both gelatin and
egg albumen cause fixation of the stain in a variable manner. Calcium deposits
stained orange red by adopting the said method.

According to Verzer and Mc Dougall (1936), the absorption of the most
of the substances including calcium takes place in the small intestine. However,
it is stated by Mitchell (1956) that the actual site of absorption of calcium is the
intestinal villus. Bykov (1958) states that the calcium salts are absorbed only in
relatively small quantities so that this is not attended by any sharp increase in
the content of calcium in the blood. The same author states that the salts of
calcium are absorbed best when they are consumed with the food. According to
him, the absorbed calcium leaves the intestine through the blood vessels and
lymph spaces, as is also evident by the present study. The present
histochemical observations reveal the presence of calcium in the submucosal
lymph spaces, blood vessels, blood capillaries, connective tissue, mucosal
epithelial cells and the muscularis of the intestinal bulb and intestine in varying
intensities. The occurrence of calcium deposits in the intestinal bulb and
intestine of the fish studied, can be explained on the basis that the dissolved
minerals in the water or mixed with the food or in a free state are taken up
during the normal course of feeding, which are absorbed and then transported
to the blood stream for the maintenance of various metabolic functions.

The occurrence, localization and distribution of alkaline phosphatase in
different parts of the digestive system of various species of fishes have drawn
the attention of number of workers (Al-Hussaini, 1949; Prakash, 1961;
Srivastava, 1966; Goel and Sastry, 1973; Sastry, 1975; Sinha, 1979; Kothari,
1983; Sinha et al., 1988; and Kozaric et al., 2004 and 2006). The aforesaid
authors have reported the presence of alkaline phosphatase in varying
intensities in different locations. It has been observed during the present study,
that alkaline phosphatase is distributed in the buccopharynx, oesophagus,
intestinal bulb, Intestine, rectum and hepatopancreas.
AL-Hussaini (1949) reported the presence of alkaline phosphatase in the basal layer of the striated epithelium of the buccopharynx of adult teleosts *Cyprinus carpio, Rutilus rutilus* and *Gobio gobio*, while Prakash (1961) in adult *Salmo* observed this enzyme in the cytoplasm and striated epithelium of the buccal and oesophageal regions and pointed out that this activity was lowest of any region of the gastrointestinal tract. Sastry (1975) observed traces of alkaline phosphatase activity in the oesophagus of *Heteropneustes fossilis* and *Cirrhinus reba*. On the other hand, Sinha *et al.* (1988) in *Mystus aor* observed intense alkaline phosphatase activity in the lining of epithelial cells, basement membrane, nuclei of club cells and submucosal connective tissue of the buccophrynx and oesophagus, while Kozaric *et al.* (2004) observed the activity of alkaline phosphatase only in the lamina propria of the oesophageal region of *Merluccinus merluccinus*. The present observations are in correlation with the findings of Sinha *et al.* (1988) and Kozaric *et al.* (2004).

In the intestinal bulb and intestine, intense alkaline phosphatase activity is observed in the brush borders and columnar epithelial cells of the mucosa, while moderate activity has been detected in the submucosal blood capillaries and the connective tissue. Similar observations have been made in the intestinal segments of various species of fishes (Goel and Sastry, 1973; Sastry, 1975; Sinha, 1979; Stromband *et al.*, 1979; Chakrabarti and Sinha, 1982; Sinha *et al.*, 1988; Kuz’mina and Smirnova, 1992; Tengjaroenkul *et al.*, 2000 and Kozaric *et al.*, 2006). According to Loyda *et al.* (1979), alkaline phosphatase is found primarily in cell membranes where active transport takes place. Intestinal alkaline phosphatase is considered to be involved in absorption of nutrients such as lipid, glucose, calcium and inorganic phosphate (Cousin *et al.*, 1987; Roubaty and Portmann, 1988; Haris, 1989; Dupuis *et al.*, 1991; Mahmood *et al.*, 1994 and Kozaric *et al.*, 2006). The present findings clearly suggest that the intestinal digestion and transport of nutrients occurs mainly in the intestinal bulb and intestine. A progressive decrease in alkaline phosphatase activity
noted in the rectum of the fish investigated is indicative of the fact that the nutrient absorption is not an important function of this region, as also has been suggested by Kozaric et al. (2006).

In the hepatopancreas, intense alkaline phosphatase activity is observed particularly in the nuclei of the hepatocytes. The occurrence of alkaline phosphatase in the liver indicates its role in the formation of glycogen through dephosphorylation process. On the other hand, highest enzyme activity noted in the intrahepatic pancreatic acini is probably related to the synthesis of zymogen granules in addition to its role in the production of various digestive enzymes. Similar observations have been held earlier (Sastry, 1975).

Acid phosphatases are widely distributed in animal tissues; prostrate, spleen and liver being three of the richest sources (Pearse, 1972). Folley and Kay (1936) and Goodlad and Mills (1957) have differentiated at least three types of acid phosphatases on the basis of their pH optima, their sensitivity to Mg$^{2+}$ ions and their relative activity towards $\alpha$- and $\beta$- glycerophosphates.

Acid phosphatase is one of the marker enzymes for lysosomes, but its activity has also been detected outside lysosomes (Chi-wei and Fishman, 1972). The results of the present investigation reveal the presence of acid phosphatase in all the tissues of digestive system of the fish studied. The mucosal epithelial cells, the lamina propria of oesophagus, the submucosal lymph spaces, blood vessels, blood capillaries, connective tissue, the nuclei of the hepatocytes and the intrahepatic pancreatic acini are observed to be the sites of intense localization.

Sinha et al. (1988) reported moderate activity for acid phosphatase in the stratified epithelial cells and the submucosal layer of buccopharynx and oesophagus of Mystus aor. However, in the investigated fish, intense acid phosphatase activity has been noticed in the stratified epithelial cells and the submucosal layers of both buccopharynx and oesophagus. Intense activity
noted may be due to their secretory nature. Regarding the intensity and the
distribution pattern of acid phosphatase in the entire intestinal tract, the present
observations are in correlation with those of Rode and Frank (1967), Sastry
(1975), Sinha (1979), Chakrabarti and Sinha (1982) and Kozaric et al. (2004
and 2006). Pronounced activity observed in the columnar epithelial cells of the
mucosa and the submucosal layer can be correlated to its secretory and
absorptive nature as is also evident from the views of Sastry (1975), Baglole et
al., (1998) and Kozaric et al. (2004 and 2006). In the hepatopancreas, strong
activity noted particularly in the nuclei of the hepatocytes may be related with
the process of secretion. Intense enzyme activity observed in the intrahepatic
pancreatic acinar cells of *Schizothorax esocinus* is in conformity with the
findings of Sastry (1975) and Sinha et al. (1988). This can be attributed to the
synthesis of zymogen granules in addition to its role in the production of
various digestive enzymes.

Lipases are enzymes of low specificity requiring only an ester linkage
for their action. While most of the workers have detected lipase in tissue
extracts of fishes (Babkin and Bowie, 1928; Mackay, 1929; Beauvalet, 1933;
Chesley, 1934; Bayliss, 1935; Ishida, 1936; Sarbahi, 1951; Fish, 1960 and Das
and Tripathi, 1991), few have demonstrated its presence histochemically in the
various portions of the digestive system of different species of teleosts (Al-
Hussaini, 1949; Sastry, 1974a, b; Swarup and Goel, 1975 and Tengjaroenkul et
al., 2000).

The results of the present study demonstrate the presence of lipase in the
intestinal bulb, intestine and hepatopancreas of the fish investigated. According
to Vonk (1937), lipase activity in the intestine is probably due to adsorption of
the pancreatic enzyme into the intestinal mucosa. However, the present
findings clearly reveal that the mucosa of the intestinal bulb and intestine is
capable of secreting lipase as evidenced by intense activity noted in the
columnar epithelial cells and the mucosal border. These observations are in conformity with the earlier findings (Sastry, 1974a, b; Swarup and Goel, 1975 and Tengjaroenkul et al., 2000). The presence of lipase in the submucosal region might be due to the possible hydrolysis of fats.

In the hepatopancreas, lipase activity is observed to be more pronounced in the intrahepatic pancreatic tissue than the hepatic tissue proper. This is in accordance with the earlier findings (Sastry, 1974a, b and Swarup and Goel, 1975). Barrington (1957) opined that the distribution of lipase in the extracts of combined liver and pancreas is not necessarily evidence of its production by the pancreas, since such activity might be a property of the hepatic tissue itself. The present observations extend support to the view of Barrington (1957).

In the present investigation, formalin fixed frozen sections were processed for ATPase activity adopting Lead method (cf. Pearse, 1972). It has been observed that the different parts of the digestive system studied reveal the presence of ATPase. In the buccopharynx, the stratified epithelial cells, the submucosal layer and the muscularis region exhibit intense activity for the enzyme, whereas, in the oesophagus the enzyme is found to occur in higher concentration in the lamina propria and the submucosa. Regarding the distribution and intensity of ATPase in the intestinal tract the present observations agree with those of Sastry (1975 and 1976) who observed intense activity of ATPase in the brush borders and the mucosal epithelial cells of *Ophicephalus punctatus* and *Heteropneustes fossilis* respectively. A steady decrease in the intensity of enzyme activity is noted in the rectum as compared to the intestine.

Sastry (1975 and 1976) reported strong ATPase activity in the cytoplasm and nucleus of the hepatic cells of *Ophicephalus punctatus* and *Heteropneustes fossilis* respectively. However, in the present study the activity is observed to be moderately associated both with the cytoplasm and nucleus of
the hepatic cells. Instead, ATPase activity is observed to be maximum in the intrahapatic pancreatic tissue. Intense distribution of ATPase in the digestive tract is suggestive of its role in the maintenance of functional integrity of the plasma membrane and in several intracellular functions (Parvez et al., 2006). Gawlicka et al. (1995) and Baglole et al. (1998) are of the opinion that ATPase in collaboration with alkaline phosphatase help in the transportation of small peptides and amino acids resulting from the action of digestive enzyme through intestinal enterocyte membranes.

5.2. Biochemistry

The biochemical studies on various fish tissues have drawn the attention of several workers (Mustafa, 1983; Gill and Weatherley, 1984; Weatherley and Gill, 1987 and Basade et al., 2006). In the present study, the total percentage of proteins, lipids and carbohydrates have been observed in the digestive organs viz. hepatopancreas, intestinal bulb and intestine of a naturally feeding fish Schizothorax esocinus. The protein content of the fish tissues studied is observed to be the most dominant biochemical constituent. Although protein plays a very important role in the fish growth and other metabolic activities, but the essentiality of lipids cannot be ignored. Lipids act as source of metabolic energy and serve to maintain the structure and integrity of the cellular membrane, besides, being precursors of bioactive molecules (Zhu et al., 2003).

In the present study, protein content of the hepatopancreas is found to be significantly higher (37.33%) as compared to that of the intestine (20.87%) and intestinal bulb (19.05%). However, no appreciable differences in the protein content are observed in the intestinal bulb and intestine in the fish studied. Similarly, hepatopancreas shows higher concentration of lipids (22.65%) followed by intestinal bulb (16.67%) and intestine (14.21%). Almost similar observations, with respect to the fishes have been made earlier (Love, 1970;
Novikov, 1971; Dorucu, 2000; Das and Sahu, 2001; Bechtel and Oliveira, 2006 and Naesje et al., 2006).

Elliot (1976) and Weatherley and Gill (1983 and 1987) observed that fishes are rich in protein reserves and poor in fat reserves. This is also supported by the present study, which shows high protein content in the tissues studied than the lipid content as the main energy sources for the fish are proteins and lipids. This may be due to the fact that the fish metabolism is well adapted to deal with diet in which proteins and lipids are high (Chellapa, 1988) and also the ability of fish to eliminate nitrogenous wastes rapidly and continuously (Dorucu, 2000). Medford and Mackey (1978) reported that endogenous proteins and lipids act as an energy substrate when considerable energy is required for spawning activity. The presence of high protein and lipid contents in the hepatopancreas of the fish studied suggest that these act as reserve nutrients for the maintenance and growth of the fishes. Several workers reported that fish tend to utilise stored body nutrients such as proteins, lipids and carbohydrates from all the body parts including liver, muscle and intestinal tract as a source of energy for growth and maturation during spawning period (Love, 1957; Halliday, 1969; Blay and Eyeson, 1982; Elisasen and Vahl, 1982; Sheridan et al., 1983; Sinha and Pal, 1990 and Jyotsna et al., 1995). The present investigations further reveal more lipid content in the intestinal bulb than intestine, supporting thereby the findings of Noaillac-Depeyre and Gas (1983).

Apart from proteins and lipids, carbohydrates are also the important biochemical constituents which serve as an energy reserve for normal growth and other metabolic activities. It also serves as a role for protein sparring effect in fishes (Hassan and Jafri, 1996). It has been observed during the present study that the carbohydrates form a minor percentage of total composition and the lowest component in the tissues studied. The carbohydrates content in the
present study fluctuates from 0.80% to 4.72%. The highest content of carbohydrate has been observed in the hepatopancreas as compared to the intestinal bulb and intestine. The intestinal bulb and intestine show insignificantly low values of carbohydrates. Das and Sahu (2001) also reported lowest percentage of carbohydrates content in the tissues of *Mugil cephalus*, *Mugil macrolepis*, *Liza macrolepis* and *Therapon Jarjua*, thereby supporting the present findings.

Chang and Idler (1960) and Bellamy (1968), reported that the carbohydrates are most readily utilized and first to be affected by the depletion. This may possibly be the reason for the lowest percentage of the carbohydrates found in the tissues studied. The carbohydrate content in the hepatopancreas of *Schizothorax esocinus* is observed to be higher than the intestinal bulb and intestine. The present findings are in accordance with the observations made by Yanni (1961), Sivakumar *et al.* (1994) and Das and Sahu (2001). According to Love (1970), the digestive tract of fish feeding naturally is presumed to be full nearly all time, so that a stream of nutrients diffuses into the wall of the said tissues and later into the blood stream. Hence, the presence of the proteins, lipids and carbohydrates in the intestinal tract of the fish studied during the present investigation may be attributed to the diffusion of these substances from the lumen into the wall of the intestinal tract.

The feeding strategy of fishes is dependent upon the enzymatic ability to digest many kinds of food (Chakrabarti *et al.*, 1995). During the present investigation, the specific activities of the different digestive enzymes namely, alkaline phosphatase, acid phosphatase and lipase have been studied. The significantly higher activity of alkaline phosphatase in the intestinal bulb than the intestine of the fish studied is an agreement with the findings of Villanueva *et al.* (1997) in *Cyprinus carpio* and Hakim *et al.* (2006) in *Oreochromis mossambicus*. The intestinal alkaline phosphatase is involved in the transcytotic
movement of lipid droplets and this role of intestinal alkaline phosphatase could be beneficial for animals to ingest high fat food (Narisawa et al., 2003). Alkaline phosphatase activity has been reported to be an indicator of the intensity of nutrient absorption in the enterocytes of fish (Harpaz and Uni, 1999; Gawlicka et al., 2000; Grewal and Mahmood, 2004 and Mozes et al., 2004) and may also be related to the fluidity of the intestinal microvillus membrane (Gelman et al., 1984).

Debnath et al. (2007) reported higher activity of acid phosphatase than alkaline phosphatase in the digestive tract of the fish, *Labeo rohita*, which is also evident from the present study. The intense activity of acid phosphatase observed is suggestive of its role in growth and nutrient absorption (Debnath et al. (2007).

The present observations further reveal higher acid phosphatase activity in the hepatopancreas than the alkaline phosphatase. This is accordance with the findings of Mawdesley-Thomas and Barry (1970), in rainbow trout. The presence of both alkaline and acid phosphatases is indicative of their role in the functional activity of the hepatopancreas.

Lipase activity in the fish studied is demonstrable at pH 7.0, which is nearer to the pH of gut fluid. Bitterlich (1985) reported 7.0 as the optimum pH for lipase activity in the stomachless filter-feeding carps, *Hypophthalmichthys molitrix* and *Aristichthys nobilis*. It has been observed in the present study that not only lipase activity is present throughout the intestinal tract of the fish but the intestinal bulb exhibits increased activity than rest of the intestine. This is indicative of the fact that the anterior intestine is probably the most important region in lipid digestion. The responsive trait and the level of the lipase activity give this region a regulatory character in the digestion of lipids (De Almeida et al., 2006). According to Reimer (1982), the lipase pattern of *Brycon cephalus*, a neotropical freshwater fish, is adjusted to the amount of lipid present in the
intestinal content. Highest lipase activity observed in the hapatopancreas suggests its profound secretory nature. This is in agreement with the findings of Sarbahi (1951) in *Carassius auratus* and *Micropterus salmoides*.

5.3. SEM

The morpho-anatomical features of the digestive tract of Indian freshwater teleosts, in relation to their feeding habits are well documented (Sinha and Moitra, 1975; Sinha, 1979; Sinha and Chakrabarti, 1984 and 1985). According to the said workers, densely coiled intestine, modification of mucosal folds and complexity of the intestinal villi are the main features associated with the herbivorous mode of feeding. It has been observed in the present study that the mucosal folds in the various regions of the digestive tract of the fish studied get modified in different ways.

The buccopharynx and the oesophagus display a well elaborated pattern of primary or major folds, which are subdivided into many secondary or minor folds. These primary and secondary folds aid in swallowing. Similar mucosal foldings have been observed in carp, *Catla catla* by Sinha and Chakrabarti (1985) and in *Serola dumerili* by Grau et al. (1992). The apical ends of the stratified epithelial cells form the mucosal surface of the buccopharynx. The apical surfaces of the stratified epithelial cells of buccopharynx and the luminal surface of the stratified epithelial cells of the oesophagus are provided with complex patterns of microridges, which increase the surface area and provide the channel for mucous transport. According to Harding (1973), the microridges on the epithelial surfaces are subjected to mechanical insult. Hence, the microridges may therefore, represent a mechanical adaptation which in the buccopharynx and oesophagus would withstand the trauma resulting from ingested materials. The presence of microridges plays a major role for anchorage of mucous film secreted by the neighbouring mucous cells over the soft mucous membrane. Therefore the microridges of the buccopharynx and the
oesophagus by virtue of their nature would seem to spread and hold mucous film secreted by the adjacent mucous cells. These observations are compatible with the studies made earlier (Sperry and Wassersug, 1976; Sis et al., 1979; Ezeasor and Stokoe, 1980; Sinha and Chakrabarti, 1985 and Murray et al., 1994). The secretion of mucous serves as a lubricant in transmitting food from buccopharynx to oesophagus thereby enabling the oesophagus to act as a transit tube for ingested food from the oropharyngeal cavity to the intestinal bulb. The secretion of mucous by the mucous cells to the exterior has been reported by several workers in various teleosts (Kapoor, 1953 and 1958; Khanna, 1964; Moitra and Sinha, 1971 and 1972; Sinha and Moitra, 1975 and 1976; Moitra and Ray, 1977 and 1979; Martin and Blaber, 1984; Humbert et al., 1984; Sinha and Chakrabarti, 1985 and 1986; Chakrabarti and Sinha, 1987; Tibbets, 1997; Park and Kim, 2001 and Podkowa and Goniakowska-witalinska, 2003). A number of workers have reported taste buds in the oesophageal wall of many teleosts (Ezeasor and Stokoe, 1980; Meister et al., 1983; Morrison, 1987 and Fishelson et al., 2004). Sis et al. (1979) demonstrated taste buds only in the cranial portion of the oesophagus of the channel catfish, *Ictalurus punctatus*. However, during the present study, no taste buds were observed in the oesophagus. This is in correlation with the observations made by Reifel and Travill (1977), Elbal and Angulleiro (1986) and Grau et al. (1992) in different teleosts.

The morphological features of the mucosal surface of the intestinal tract in *Schizothorax esocinus* as revealed by present SEM study are more or less similar to other teleosts (Sinha, 1983; Sinha and Chakrabarti, 1985 and 1986; Chakrabarti and Sinha, 1987; Kapoor and Khanna, 1994 and El-Shammaa et al., 1995). It has been observed that the mucosal folds in various regions of the intestine get modified in different ways. The major mucosal folds, noted to be very high, consist of zigzagging pattern that course along the long axis of the entire intestine and form deep cavities between them. The major mucosal folds
along with the minor ones around the cavities not only serve to increase the total surface area but also retain ingested food for longer periods in the stomachless fish studied. The deep cavities in the intestinal region are an adaptational feature meant mainly for storage. It is well known that the primary function of this region is absorption and secretion. Never the less, partial retention of the semi digested food also occurs while the same passes though the zigzag course of this region. The zigzagging pattern of the major mucosal folds has been reported by Mc Vay and Kann (1940) and Caceci (1984). According to Curry (1939), the tops of the mucosal folds in the intestinal bulb are flat, round or pointed. However, during the present study, the tops of the mucosal folds in entire intestine are flat and rounded. Caceci (1984) also observed flat and round folds in the intestinal mucosa of goldfish, Carassius auratus. The luminal surface of the entire intestine in Schizothorax esocinus is lined with well-developed columnar epithelial cells. The luminal plasma membrane of the columnar epithelial cells of the anterior portion of the intestine is sculped into microridges. Sinha (1983) and Sinha and Chakrabarti (1985) report that the microridges represent a mechanical adaptation in the intestine. According to various authors, the microridges are not only restricted to the intestinal surface but have been found to occur also on the epidermal cells of the body (Hawkes, 1974; Lanzing and Higginbotham, 1974; Harris and Hunt, 1975; Hunter and Nayudu, 1978; Bereiter-Hahn et al., 1979; Sinha, 1983 and Whitear, 1990), epithelial cells of the gills (Rajbanshi, 1971; Olson and Fromm, 1973; Mattey et al., 1979; Olson, 1995 and Eiras –Stofella and Charvet –Almeida, 1998) and the stratified epithelial cells of buccopharynx and oesophagus (Sperry and Wassersug, 1976; Mallatt, 1979; Sis et al., 1979; Clarke and Witcomb, 1980; Ezeasor and Stokoe, 1980; Sinha, 1983; Humbert et al., 1984; Sinha and Chakrabarti, 1985; Mac Donald, 1987; Grau et al., 1992 and Murray et al., 1994). The presence of microridges on various types of epithelial cells in teleosts probably indicates certain common function.
The rectum reveals irregular mucosal foldings that increase the surface area of the rectal mucosa. The rectum plays very little role in food storage, digestion and absorption as evidenced by the low mucosal folds as well as the absence of well-defined cavities and the minor mucosal folds, thereby bearing a close relationship with its functional aspects. A thin film of mucous secretion has been observed in the rectal mucosa, which aid in easy defecation. The occurrence of few mucous cells in between the epithelial cells of the various regions of the digestive tract of various teleosts has been reported by different workers (Khanna, 1964 and 1968; Moitra and Sinha, 1971 and 1972; Sinha and Moitra 1975 and 1976; Sinha, 1975 and 1978 and Park and Kim, 2001). According to the above-mentioned workers, the presence of copious mucin discharged by the mucous cells in the different regions of the alimentary canal of various teleosts is an adaptation for adequate lubrication of food for facilitating conduction posteriorly along the alimentary canal.