INTRODUCTION

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Libbe Henrietta Hyman (1967) one of the worlds leading invertebrate zoologists with her extensive efforts for 27 years have rightly claimed that the digestive system of prosobranch presents so many variations which are mainly in co-relation with feeding habbits. Due to this, uniform terminology to the system is very difficult to suggest. A general plan of histology and histochemistry can be traced by refering her review published by Mc graw-Hill publication in the year 1967.

The mouth is generally situated on a level with tentacals and eyes. In marine carnivorus prosobranches the snout is prolonged into proboscis which is recognisable into two sorts, acrembolic and pleurembolic. The former is completly invaginable while later shows only the basal part of the proboscis which is invaginable. Acrembolic type of proboscis is a characterstics of cypraea and other monotocardian families like strombidae, capulidae, calyptracidae etc. Accomplishment of four longitudinal muscles with this invagination in cypraea was described into shorter and longer pairs. (Amaudrut, 1898 and Rau, 1934) Little attention has been paid to proboscis since the work of Bouvier (1887) Oswald (1894) and Amaudrut (1898) except the study of pleurombolic type Tonna (Webber, 1927). The outer circular and inner longitudinal strata. In Tonna, (Webber,1927)reported three layers of circular fibers of varying direction preceded by a layer of connective tissue and followed by thick longitudinal layer. In Oliva the proboscis wall consist of cuticle, epidermis, thin circular muscle layer containing numerous subepidermal glands and inner circular muscle layer (Kuttler, 1913). The mouth is round or vertical slit placed centrally at the tip of the snout. The mouth is usually bordered by a histologically distinct area termed lip, which ~~is~~ generally consist of tall slender epidermal cell interspersed with gland and neurosensory cells. The epidermal cells are provided with cuticle, sometime quite thick and is subtended by retractor muscle bands. Mouth leads into the smaller buccal cavity which is commonly lined with cuticle, often quite thick at least in some areas it covers the columnar epithelium which contains mucocytes or entirely by mucocytes. Beneath the columanar layer there is circular muscle fiber layer acting as sphincter they are preceded by longitudinal muscle layer. Buccal pouches of glandular nature are seen in some primitive forms like Haliotis, Patella, Pleurotomeria, Acmaea, Lottia, Fissurella, Turbo, Nerita, Ampularis etc. Posteriorly buccal cavity continues with pharynx, most of it is occupied by a mass consist of redular apparatus. Buccal mass, buccal bulb, phryngeal mass and odontophore are the different terminologies for it. It is extremely complex assemblage of muscles that supports and operates the redula. In lower prosobranches (Patella, Lottia, Haliotis etc.) also in Trochus, Turbo, Nerita, Cypraea and taenioglossan in general the dorsal part of pharynx or the beginning of the oesophagus bear a pair of pouches called oesophageal pouches. They have typical acinous structure. They appear to produce a mucoid secretion which is thought to be lubricant in nature (Graham, 1941 and Carikker, 1943). From the redular mass in the pharynegal floor there protrudes theredular sheath which is often very long. Oesophagus proceeds posteriorly above the redular mass. The lining of the oesophagus is frequently with short to tall ciliated columnar epithelium. It consists of mucocytes supported inwordly by connective tissue and muscle fibers. In Tonna the epithelial layer is liberally provided with mucocytes (Webber, 1927). Sometimes the oesophagus at its beginning presents an expansion variously called pyriform organ, crop, jabot, pharynx of Leiblein and oesophageal bulb. The interior of bulb is more or less folded almost transverse in its orientation. Clothing of these folds is with a columnar epithelium which are often tall and ciliated in nature. In Cypraea, the bulb is posteriory located. In Haliotis and Patella the oesophageal bulb has highly folded wall (Graham, 1932). Oesophagus course posteriorly and enters into stomach called forestomach or proventriculus has been noticed first by Fischer (1904) in Lottia, also in Pattela cellana and related forms by Thiem (1907). The stomach may be obvious sacciform expansion or a tubular continuation not definitely delimited externaly from the oesophagus. In the first case the oesophagus usually enters the middle or posterior part of the stomach and the intestine leaves anteriorly. Stomach is not muscular in having thin circular and longitudinal coats outside epithelium except in Pila where it is thick circular coat (Prashad, 1925). The stomach is reduced in size and is of simpler construction in carnivorus prosobranch or those ingest larger particals. The wall of stomach lined internally by tall epithelium usually ciliated in some areas, provided with cutical in others. In many prosobranches the stomach has an anterior evagination termed style sac, that contains a rod known as the crystalline style. According to Young (1930, 1932) it is associated with herbivorus diet.

A variously called gland liver, midgut gland, hepatopancreas, and digestive gland is of dark coloured brownish structure constitute the greater part of visceral mass. It is always intermingled with gonads. Primitively the gland is said to be of two separate symmetrical lobes, but usually it is a single lobulated mass of compound tubular or acinous nature. The duct or ducts arising from it open into stomach at oesophageal enterance. The ducts are lined with columnar ciliated epithelium.

Histological pattern of midgut gland cells has been repeatedly investigated but much disagreement exist because of different stages of secretion have been mistaken for distinct cell types. Barfurth (1883) and Frenzel (1885, 1886, 1893) described three types of cells in midgut gland of prosobranch namely ferment clubs, granular cells and calciferous cells. Calciferous cells are of common occurrence but are absent in *Patella* (Graham, 1932) *Buccinum* (Dakin, 1912) *Scutus* (Owen, 1918) *Oliva* (Kuttler, 1913). The midgut gland of *Haliotis* lacks separate calciferous cells but calcium spherules occure in both of the cell types present (Crofts, 1929 and Manigaut, 1939). Thiel (1953) recognizes three stages of calciferous cells and identified the ferment cells of earlier author as the third stage. The calciferous cells are generally shorter and broader than the other cell types and contain in their somewhat fluid interior, granules or spherules that gives histochemical test for calcium. The calcium apparently exists as calcium phosphate but when calcium bodies are dissolved by acid, an amorphous material remains. Hence the calcium salt is bound to protein (Thiel, 1953 and Hirst, 1917). Thiel (1953) is of the opinion that there is one cell type other than calcium cells, performs the function of enzymatic secretion and absorption of digested material. These cells are tall, cylindrical with granular free ends and basal nuclei. However Morton (1951) in <u>struthiolaria</u>, Cleland (1954) in <u>Valvata</u> and and Owen (1958) in <u>Scutus</u> indentified another cell type called excretory cell. These cells are tall cylendrical drak and contains granular spheres. Alpers (1931) in <u>Conus</u> recongnized two cell types in the midgut gland. These are calciferous and enzymatic absorptive cells. Similar cell types were reported for <u>Pomatias</u> by Urban (1932), Graham (1932), <u>Patella</u>. Croft (1929) and Manigaut (1939) have decribed two cell types in <u>Haliotis</u> which are young and aged stages of the same cell. According to Manigunt the young cell contain spherules rich in iron at basal region of nucleus and granules rich in calcium distal to nucleus. Older cells especially in winter show large bodies composed of calcium associated with a mucoprotein. Cleland (1954) in valvata found calciferous absorptive and excretory cells. Owen (1958) reports absorptive excretory and flagellated cells in <u>Scutus</u>, Frazo (1957) regards all cell types in the midgut gland as derivatiaes of single type and identifies the granular bodies as composed of mucoproteins or mucopolysaccharides.

Intestine gets its origin from stomach and runs anteriorly. In lower prosobranches it may be thrown into loops (<u>Haliotis</u>) or coils (<u>Patella</u>) while in higher ones it usually runs a more or less streight course to the anus. It is frequently of same diameter but may be differentiated into slender proximal small intestine and wider distal large intestine. The said condition is observed in *Lambis* and other strombidae. A widen terminal part may be named as rectum. Intestine in the mantle after emerging from visceral mass is at the roof and ends as anus at free edge ofm mantle. Intestine in mantle either passes through pericardium (Haliotidae, Fissurellidae, Trochidae, Neritidae, Turbinidae) or runs along the edge of the pericardial cavity. The lining of intestine is either with cuboidal or with ciliated columnar epithelium. Near the anus the cilia may be replaced by cuticle. The interior of intestine ise either smooth or thrown into longitudinal ridges supported by connective tissue. The epithelium is subtended by connective tissue containing some muscle fibers. Intestine near the anus is provided with an anal gland in number of prosobranches and gives dark colour. Such gland is seen in Fissurellidae (Pelseneer, 1898 and Spelter, 1928) Muricidae (Fretter, 1946) and Naticidae, - *Oliva* (Kuttler, 1913). *Circulus* (Fretter, 1956). The epithelium consists of one kind of cell, ciliated and contains brown granules which are discharged into the rectum for elimination.

Apart from the review by Hyman (1967) there are several contributions regarding the morphology, anatomy, histology and to some extent on histochemistry of digestive tract of gastropod prosobranch. Those can be summerized briefly as under.

A histochemical study of mucus secreting cells in the epithelial lining of alimentary tract of Marisa Cornuarietis has ~~been~~ revealed 6 types of glandular cells secreting carbohydrate rich substances. These mucus cells are designated on morphological grounds as fusiform, club shaped, ovate, globate, saccular and elongated conical. Their histology together with their distribution is different in different organs. By using various recently developed techniques 4 types of mucosubstances are found to be producedby these 6 cell types. Fusiform and club shaped cells produced neutral mucosubstances showing moderate to weak PAS activity, ovate cells serects strongly PAS reactive neutral mucosubstances with lack of associating basic protein. Goblet cells and saccular cells are PAS unreactive shows acidic, sulphated mucosubstances or sulfomucins, elongated conical cells are highly PAS reactive shows weakly acidic sulfomucins (Demian, et al. 1972). Histology of the digestive system and histophysiological changes during digestion are described in 6 spp. of Atalanlidae namely Atalanta, inflata, A. fusca, A.lesueri, A.peroni etc. Morphological features like presence of tubular salivary glands and disgestive glands but absence of oesophageal and rectal glands are described. Total absence of mucous cells in alimentary canal is also mentioned. Several unicellular glands are seen in the buccal wall and anterior part of oesophagus. Glandular activity is minimal in stomach and absent in intestine, (Martoja, et al. 1976). Carbohydrate digestion in the snail Tegula funebralis has been studied and the major enzymes, amylase in buccal cavity, stomach, digestive gland, spiral caecum, thin and thick hindgut, laminarase and alginase in buccal cavity, oesophagus, stomach, and digestive gland and cellobiase in salivary glands, spiral caecum and hindgut are reported. Some carbohydrate hydrolysis appears to be due to the bacterial action (Berrie et al, 1965).

Carbohydrate metabolism in the fresh watered prosobranch snail Viviparus bengalensis wasstudied by injecting insulin. Insulin lowered the haemolymph glucose level and recovery set later. Increased doses of insulin produced increased hypoglycemia in foot muscle and hepatopancreas. Glycogen content was increased (Kulkarni and Utkar, 1982).

Injection of three ganglionic extract, visceral, pleuropedal and cerebral into P. globosa effects on various neutral and phospolipid fraction of hepatopancreas, albumen gland and veginal tract. Injection of visceral ganglionic extract caused a profound increment in lipid content, suggesting the existance of lipogenic active principal (Ramamurthi et al., 1982). Feeding habbits, feeding mechanisms and histological features of digestive tract in Cellana rediata were observed and described (Rao, 1975). According to him the animal feeds when submerged in tide water. Redula helps for scrapping the rock surface. He divides gut into foregut, midgut and hindgut. Absence of crystalline style and presence of amylase was detected in foregut. No enzyme activity was seen in midgut and hindgut. Digestive gland was the source of digestive enzymes which acts on starch, sucrose, lactose, glycogen, maltose, dextrose, gelatine and caesin. Absorbtive and secretory cells are indentified into digestive gland. Digestion was said to be intracellular. Peculiar feeding was observed in Maxwellia santorosana. The molluse in aquarium drilled a tapered hole into upper valve of the pelcypod Choma arcana. Drilling lasted for 9 days. The animal preys in rocky intertidal to subtidal areas (Wicksten, 1980).

Contribution for redula, its morphology, mechanism, orientation is comparitively immense in molluse literature. The presence of reducing secretions and occurance of perioxidase activity in the epithelium of the redular teeth suggest that quinone tanning (hardening) of the protein component of the redular teeth occurs in two steps (Decros, 1968). Bibliographic description of redulae of about 100 publications has listed (Schilder, 1971). External anatomy of Cypraea fulfoni and C. capensis with redular morphology has been discussed (Kilburn and Donald, 1973). Further notes on redular teeth of 7 species of Indionesia are discribed and discussed pointing out the variations as regards to strucure and form. (Soemodihardjo, 1973). Asymmetry is fundamental adaptive feature of rhiphidiglossate archaegastropode redula. This was studied in 5 marine achaegastropode superfamilies Pleurotomariacea, Fissurellaceea, Cocculinacea, Trochacea and Neritancae. Possible functional evoutionary trend is traced (Hickman, 1981). Redular anatomy histology and histochemistry were studied in *Conus striatus*, *C.marmorus*, *C.flavides*. and *C. lividus*. Aminoacids, proteins, carbohydrates, mucosbstances and inorganio ions were tested. The significance of positive staining of redular chitin by the PAS reaction is discussed (Marsh, 1978).

Histological description of the different structures of pharyngeal bulb in the snail, *Ampularia canaliculata* were emphasized. Its morphological and secretary characteristics changes according to the zonal function and especially in relation to the development of masticatory structures are worked out (Catalan *et al*., 1981). With the inclusion of scanning eletron mircrosoopy the redulae of 33 spp. of neogastropoda were described. The taxonomic value of redula morphology in prosobranch systemics as advanced by Troshel (1886) were confirmed and discussed using the new methods (1979). Taxanomic revision with reference to secondary sexual Characters found in dimorphic *Drupella* with the use of scanning electron mircoscopy. Here rechidian teeth of male are larger than females. the juveniles exhibit no redular sexual dimorphism, having thin and fragile rachidians as the adult females (Fujoka, 1982).

The histology and histochemistry of salivary gland, snout and secondary salivary glands in Conus flavides and C. livides was investigated. The salivary gland secretion was composed of polysaccharides and proteins. The snout gland secretes sulphated acid mucosubstances (Marsh, 1972) Cytochemical analysis showed three cell types mucous, granular and mixed cells in Cyclope nerita and Amiclina tinei salivary glands. The cytochemistry was discussed in relation to habitat and feeding (Minniti, and Maria, 1982) The buccal mass of the snail Theba pisama was studied by using several histological and histochemical techniques. Account of epithelial cells producing mandibles and those forming redulary teeth was presented (Roldan and Cosin, 1979). Histological observations on the salivary glands of Thias bufo (neogastropoda) showed the presence of two types of alveoli mucous and serous alveoli. The histochemical test reveled the presence of acid sulphated and neutral polysaccharides, sailomucins, hyalomucins and glycoproteins (Bhanu et al., 1982). The crystalline style of the gastropod Telescopium telescopium consists of series of the stacked cones which appear as concentric rings and not spiral structure. The style is permanent and it is composed of protein and mucosubstances. It probably produces digestive enzymes (Alexander and Rae, 1974). A carnivorus mesogastropod Polinices lewsis preys upon bivalve. The anterior region of oesophageal gland secretes masked precursor of trypsin activated proteolytic enzyme. It is used for extra organisimic digestion of the prey. The reminder of the gland contain amylase, glucosidase, esterases etc. The gland may be absorptive and secretory. Gastric morphology is simple and has significant role in protein digestion. Alkaline proteinases are low in digestive diverticuli which are important site of intracellular digestion (Reid and Friesen, 1980) In the intestinal mucosa of <u>Buccinum undatum</u> epithellial cells found containing small granules appearing like secretary granules of endocrine cells of higher vertebrate. These cells corresponds with B cells observed in pancreatic tubules. It is proposed that these cells represents the site of insuline production in <u>B. undatum</u> (Boquist, 1972). Insuline like substances was demonstrated in the cells of intestinal epithelium of mollusk (Kasakov, 1978).

Four cell types thin cells, digestive cells, calcium cells and excretory cells are found in the digestive gland tubule epithelium of <u>Helix aspersa</u>. The cells are phagocytic and absorptive. Excretory cells are degenrate calcium cells and exctete large granules of lipofuschin (Summer, 1965).

The digestive gland of <u>Viviparous bengalensis</u> does not show prominant rhythumicity in enzyme secretion during the post breeding period appears to be corelated with food habbits and feeding behaviour (Biswas and Ghose, 1969). The absorption of the injested food occurs in the digestive gland of marine gastropod of Nassarius spp. was observed histologically by feeding the food with reagents like carmine particals. Apical concentration of the reagent at the lamina of the tubules confirms the process of absorption by lamina cells of tubules (Mclean, 1971). Studies on phosphatases in digestive gland of the snail, Helix pomatia has revealed it higest activity at PH 4.0 in the presence of PNPP (disodium - p - nitrophenol phosphate) and pheny phosphate. The appearance of the activity peaks both on acid side at PH 3.0 and 4.02 and on alkaline side at PH 7.5 and 8.5 is suggestive of different phosphatases probably coming from digestive gland cells with different physiological functions (Pilc et al., 1976). Ferritin labelled food reaches to lumen of digestive gland and is absorbed by digestive cell, lining the gland. They form pinocytotic vesicles indicates a site of intracellular digestion in Nassarius tegula (Mclean and Holland, 1974).

Three types of the cells are present in the tubules of digestiveg gland of marine prosobranch Maoriorygta monoxyla. These are digestive cells - involved in endolytic uptake of food material; crypt cells - secrete glycoproteins probably enzymatic in function and vacuolated cells without any established function either secretary or excretory. The tubules undergo a cycle of digestive activity not related to the tidal cycle as in some marine mollusca but probably is an indirect result of feeding regime (Nelson and Morton, 1980). A histological and histochemical study of the foregut and midgut gland reveled the presence of club shaped epithelium. Two types of the cells secretary and absorptive were recognized. These glands are loaded with rich quantities carbohydrates, basic proteins, protein bound aminogroups, sulfahydrals and moderate quantities of aminoacids like tryptophan and trypsin (Bhanu _et al_. 1982). The effect of increased doses of cortisone on tissue glycogen and haemolymph glucose level in fresh water prosobranch snail _Viviparus bengalensis_ was studied. Increase in the foot muscle glycogen content and heamolymph glucose level is due to the mobilization of excess glycogen from hepatopancrease on administration of 0.01, 0.02, 0.04 ml of cortisone (Kulkarni _et al_., 1982). Histological features of the digestive gland tubules in _Pila viren_ ~~are~~ consist of two types of cells namely digestive and Ca excretory cells. Former are columnar and vacuolated and carry out function of scretion and absorbtion while latter are pyramidal, nonvacuolated serve as reservoir of Ca and carry out excretion. Mucus cells are interspread within and seems to secrete neutral and acid mucosubstances. (Devi _et al_., 1982).

SIGNIFICANCE OF PRESENT INVESTIGATION

While scanning through the available literature of mollusca, keeping the gastropod - prosobranch - cypraea in mind it was made certain that most of the studies on this animal were done on taxonomic direction. Majority of the literature deals with the discovery of new species and its systematic allotment in the phylum, wherein various shell and redular features are considered. Very little information can be traced out about histological and histochemical knowledge of the animal. This provoked the mind to undertake the project on the gastropoda - prosobranchiata.

CHOICE OF THE ANIMAL

Cypraea arebica arebica is the mollusc considered for the present investigation because it is easily obtainable from the sea shore at the intertidal zone in an adequate number through out the year. Comparatively large size of the animal is another important feature. Thirdly the detailed investigation on mucosubstances in the digestive tract of animal and its possible role in the physiology of digestion are still uncertain. Fourthly the animal is neglected for study may be due to its hinderance below the intertidal rocks. These view points stimulated this laboratory to make the investigations on the mucosubstances of various organs of the digestive tract of Cypraea arebica arebica.

CHOICE OF TECHNIQUES

As it is intended to have a detailed study of the nature of the mucosubstances present in different cellular sites in the digestive tract from buccal mass to rectum and characterize them histochemically recent and well established histochemical techniques were employed. They includes PAS reactivity, modification to PAS reactivity following diastase digestion and phenylhydrazing pre treatment, alcianophilia at different PH levels, aldehyde fuchsin staining, sequential techniques such as AB PH 1 - PAS, AB PH 2.5 - PAS, AF-AB PH 2.5, metachromasia at various PH levels, critical electrolyte concentration, methylation, saponification, acid hydrolysis enzyme digestion such as hyaluronidase digestion, neuramidase digestion and pepsin digestion. These histochemical techniques illustrate the tissue and cellular localization and are well suited over biochemical techniques though they give reliable data from quantitive point of view. The histochemical techniques employed in present investigation assures constant staining timing throughout the work and the difference if any in the intensity of staining was taken as reflection of difference in the concentration of the different types of mucosubstances.

CRITICAL EVALUATION OF THE OBSERVATION

Critical analysis of the results obtained in the present investigation on the digestive tract of _cypraea arebica_

arebica was done in relation to -

1. Histology of various organs such as, Buccal mass, preoesophagus, oesphageal bulb, post oesophagus stomach, intestine and rectum.
2. Histochemical characterization of the mucosubstances in different histological parts in different organs of digestive tract.
3. The distribution of mucosubstances in different histological parts of digestive tract.
4. The results obtained were compared to the existing literature on molluscs in general and gastropode prosobranch particular in order to find our the differences or similarities if any.
5. To find out cellular specilization if any in a given organ of digestive tract.
6. To project the idea about functional significance of mucosubstances in the various organs of the digestive tract based on circumstantial evidences.