CHAPTER ONE

I N T R O D U C T I O N

REVIEW OF THE LITERATURE, REASONS FOR UNDERTAKING THE PRESENT INVESTIGATION AND PLAN OF THE PROPOSED WORK

I N T R O D U C T I O N

Molluscan digestive tract is known to contain maximum B-glucuronidase activity in the entire animal kingdom and this enzyme has been extensively studied in the digestive gland, crop fluid and other parts of digestive tracts. At a comparative level various enzymes have been exhaustively studied in digestive tracts in invertebrates. Wherein they have been related to similar lysis and digestive phenomena. In recent years the mucopolysaccharides play various roles in relation to life in general. These substances have been studied in vertebrates as in respiratory tract, nervous system, kidney, salivary glands, lingual glands, gonads and associated glands in the reproductive tract in this laboratory (Nalavade, 1975; Pawar, 1976; Vibhute, 1981 and Fartade 1981). Similarly the mucosubstances in the reproductive organs of the invertebrates also have been studied in this laboratory (Nanaware, 1975; Varute and Nanaware, 1972; Nanaware and Varute, 1975 and Gonjari,1983). A critical review on the physiology of digestion along the digestive tract in molluscs have been investigated by several research workers. Though the structural aspects of the digestive organs have been investigated satisfactorily the metabolic aspects like mucosubstances are comparatively less understood. The present work was therefore, undertaken to get a clear picture of this important metabolice in the digestive physiology of a marine slug, Oncidium verraculatum . Some idea of the work done on histology and histochemistry in alimentary tract of different gastropods will be obtained from the following brief review.

A) Review of the existing literature on anatomy, morphology and histology of the alimentary tract of molluscs :

A glance at the existing literature on the alimentary tract and associated glands of molluscs reveals that there are several reports on the anatomy, morphology and histclogy. Recently some of the regions of the alimentary tract have also been studied by using electron microscope and scanning electron microscope.

i) Buccal mass :

Although the alimentary tract and associated glands have been studied from several molluscs from the aforementioned points of view, the buccal mass was the focal point for such studies. The morphological details and in some cases the functional aspects of different structures of the buccal mass have also been investigated elaborately. Crampton (1969) studied functional anatomy of the buccal mass of Onchidoris bilamellata and reported that about 25 buccal muscles operate the odontophore and buccal pump during feeding. The protraction of the buccal apparatus was found to be passive one and was dependent on increased blood pressure within the head and edontophore which helps in protruding the radula. Rose (1971) studied the buccal mass of a Nudibranch, Archidoris pseudoargus and described in detail the nomenclature for the various muscles operating the buccal apparatus. The structure of the buccal mass in Planorbarius corneus was described in detail by Hembrow (1973) together with the comments on its operation during the feeding. Graham (1973) has given a detailed account of muscular anatomy of the buccal mass in several species of prosobranch and amphineuron molluscs. From the observations, Graham (1973) deduced a basic muscular organization of buccal constrictor and dilator, protractors and retractors of the odontophore, protractors and retractors of subradular membrane and on approximator muscle of the supporting cartilage. Histology of the several regions such as peristome, jaws, epithelial lining of the buccal cavity radular sac, radula, odontophoral cartilage, buccal muscles and epithelial connective tissue of the buccal mass of the snail, Bulinus truncalus has been described by Lutfy et al. (1974).Rolden and Diazcosin (1975) studied the buccal mass of a gastropod, Theba pisama with regard to a detailed histological account of the epithelial cells producing mandible and those forming the radular teeth.

ii) Radula :

The structure of radula and radular apparatus has extensively been studied in the past for several gastropods (Hoffman, 1932; Brown, 1934; Marcus, 1955; Lamche, 1956; Starmuhlmer, 1956; Cate, 1967). In Opisthobranchs, the radula is not of a definite type and hence it has nod value from the classification point of view. Its structure varies from species to species. Awati and Karandikar (1948) found that the radula of Onchidium consisted of numerous transverse rows with about sixty minute teeth in each row. Recently Shimek (1975) reported on the radula and venom apparatus of Oenopota levidensis from the morphological, histological and functional points of view.

Radwin and Wells (1968) carried out a comparative study on the radulae of 11 species of muricid gastropods. They concluded that there exists a relationship between the feeding habits, and the type of the prey and the radular morphology of these species. They further reported that the radular morphology has some importance in the classification of muricid gastropods. In the physiological experiments the buccal muscles were kept in isolation for several hours by Hill (1972) and found that the contractility of the radular protractor did not decline for 5 days of hourly stimulation or over one day of stimulation at 10 minute interval.

Walsby (1975) described the mechanism of odontophore protrusion in a marine limpet, _Trimusculus reticulatus_. In this limpet, there are no jaws, salivary glands are vestigial; the radula is very small with delicate minute teeth and odontophore is large and has markedly ridged surface for mucus ingestion. Runham and Thornton (1967) studied the wear sustained by the teeth of highly mineralized radula of _Patella vulgate_ and the unmineralized radula of _Agriolomax reticulatus_ with the scanning electron microscope. In similar studies by scanning electron microscopic observations by Solem (1973) on the radular teeth of several pulmonate supported the view of convergent evolution of the cusp structure and function of Enidae and Pasfulidae and basic internal mechanism in several endodontoid land snails.

Boyle (1975) studied the ultra-structure of the subradular organ of the chiton, _Lepidochiton cinereus_. The single layered epithelium consistes of three types of cells - Viz. microvillus, ciliated and mucus secreting cells. He further reported that the fine structure is consistent with a chemosensory function for the subradular organ. Recently Fujioka (1982) reported on the sexual dimorphism in the radulae of few species of _Drupella_ which was found to be taxonomically important. He carried out the studies by scanning electron microscope and reported that the rachidian teeth of the males of D. cornus, D. fraga, D. dealbata and D. concatenata were larger in size, thicker and more massive in shape and darker in colour than those of the females.

iii) Salivary glands :

Driscoll (1972) observed small salivary glands in Cerithidea californica and Batillaria cumingi. Lutfy et al. (1973) distinguished two types of cells viz. cuboidal epithelial cells and secretory cells in the ducts of the salivary glands of Bulinus truncatus. Earlier Kulkarni (1972) found two types of cells in the salivary glands of a land slug, Laevicaulis alte. Rajyalakshmi-Bhanu et al. (1981 b) studied the histology of the salivary glands of Thias bufo and reported on the presence of two types of alveoli viz. mucous and serous. Minniti and Ricca (1980) have recently identified three types of cells : mucus, granular and mixed in the salivary glands of two species of molluscs, Cyclope nerita and Amyclima tinni. Matus (1971a) studied the posterior salivary glands of two species of Octopods, Eledone cirrosa and octopus vulgaris, wherein two distinct epithelia are present viz. type A and type B. The type A epithelium consisted predominently of columnar cells, whereas the type B epithelium consisted of three types of cells.

iv) Gastrointestinal tract :

In the last few years the attantion has been paid to the morphological, antomical and histological studies of gastrointestinal tract of molluscs. Morphology and histology of the alimentary tract of a rock chiton, Acanthopleura spinigera were investigated by Greenfield (1972). He found that the esophagus has a pair of thin walled lateral pouches (the salivary or sugar glands) empting into the stomach. The folds of capacious stomach were almost obscured by the large digestive gland, over which the intestine was coiled. Histologically the gut consists an outer layer of connective tissue, an inner muscular layer and a ciliated epithelium which varies in thickness from region to region. Sheridan et al.(1973) have studied the alimentary tract of seven species of Turridae. They reported that the stomach which has only retained the typhlosole as an ancestral structure. The digestive tract of a pulmonate slug, L. alte has been studied by Kulkarni (1972). He pointed out that the digestive diverticula formed two unequal lobes, exhibited two types of cells (liver cells and calciferous cells). The anatomy and histology of the digestive system of Parreysia corrugata were studied by Lomte (1972). He described the digestive diverticula which open into the stomach contain the group of darkly staining cells. He further noted that the lumen of the mid-gut was divided into 2 unequal chambers, one for the crystalline style and the other served as a passage for food. The alimentary tract of the two species B. cumingi and C. californica was studied by Driscoll (1972). He found that there was no accessary esophagial organs but the digestive diverticula exhibite a large duct. He further observed that C. californica has a long crystalline style, while B. cumingi passes a short style. He suggested that there is a relationship between the length of the style and composition of its food. Lateron Arnould (1973) studied the morphology and histological structure of the gastric shield of bivalve, Zyrphea crispata. He described the gastric shield of Z. crispata is formed by the gastric epithelium and composed of a non-sclerified chitinoprotein matrix. Theanatomy and histology of the alimentary tract of Scolytus multistriatus have been studied by Buker and Estrin (1974). They came to the conclusion that the mid-gut diverticula is the centre for regeneration of the midgut cells. Further they observed, the midgut epithelium and proventriculus facilitate the rapid uptake of nutrient from food. The histological structure of the alimentary tract and its changes during the course of digestion were studied in two species of cephalopods, Bledon cirrosa and Illex illecebrosus (Boucher-Rodoni, 1976). He noted that the anterior part of the digestive tract containing a cuticular layer is not concerned with digestion. Further he observed, the digestion took place in stomach with the help of digestive enzymes from the digestive glands. Fretter (1977) studied the feeding mechanism of *Volvarina taeniolata* and described that during the feeding the proboscis probably punctures an arthrodial membrane, to get the food. He further pointed out that the digestion occured in the esophagus so that the stomach was reduced. Recently the variability of the tubule types within the digestive gland of different molluscs, *Mercenaria mercenaria*, *Ostera edulis* and *Mytilus edulis* have been studied by Robinson *et al*. (1981). They identified and described four tubule types in the digestive gland of these bivalves. Anisell (1981) examined the water filtration rate in the two bivalves, *Donax Sera* and *Donax sordidus*. He further noted that the difference in the filtration rate was due to the differences in distribution of 2 species on beaches.

Lateron the research workers paid their attention, to study the cellular morphology and physiology. The digestive gland of *Patinopecton yessoensis* was studied by Suzuki *et al*. (1968) using the microscopic techniques. They found that the epithelium of the tubule of the digestive gland consisted three types of cells *viz*. basophil, secretory and fat cells. They further observed that fat cells swelled, when the temperature of the sea water rose; concomitantly the secretory and basophil cells exhibited atrophy.

Mequiston (1969) studied the histological structure of the digestive diverticula of bivalve, *Lasaea rubra* and described 2 types of cells (digestive and secretory). Kanwar and Jassar (1970) have studied the morphology and cytochemistry of the digestive gland cells of a land snail, *Euplecta indica*. The gland consisted of blind tubules, embeded in the inter tubular tissue. The tubules showed 3 cell types *viz*. digestive cum excretory cells, calcium secreting cells and undifferentiated cells. The digestive cum secretary cells in the digestive phase were tall and columnar in nature, filled with secretory granules. The nature of these granules was mucopolysaccharide-protein complex. Microscopic studies of the intestinal epithelial cells of *Pomacea ureceus* has been studied by Paceco and Scorzah (1971). They found that during the aestivation cycle, the layer of the epithelial cells showed an increased number of mucoid cells undergoing diverse stage of secretion. They further noted that a low rate of autophagic process characterized by the presence of various kinds of lysosomes. The epithelial lining of the digestive tubules of marine bivalve, *Cardium edule* was studied by Owen (1970). He described three kinds of cells (digestive, secretory and flagellated) in the epithelial lining. Further he described that the digestive cells varied from cuboidal to columnar in nature and possessed distinctive Golgi apparatus with characteristic intracisternal membranous elements. They were capable of ingesting exogenous material from the lumen of the tubule.

The digestive gland of B. truncatus was bilobed tubuloacinar type, with two main glandular cells viz. digestive and excretory. The digestive cells were dominated the epithelial lining of the gland and exhibited three different forms which may reflect three successive phases of physiological activity. On the other hand, the excretory cells were small in number and their cytoplasm filled with excretory globules (Luffy, 1973). Similar type of studies was carried out by Boghen and Farley (1974) on Littorina sazatilis . They identified and described 2 types of cells (digestive and secretory) in the epithelium of the digestive gland. They further observed that there is a close relationship between the digestive activity and the periodicity of the tidal cycle.

The histology of the digestive tract along with enzyme activity in the common limpet, Cellana radiata has been studied by Rao (1975). He reported that histologically the midgut is divided into five regions, and no enzyme activity was detected in this region. The digestive gland contained 2 types of cells, a common absorbing cells and other secretory cells. He further described, the intracellular digestion occurred in the common absorbing cells. According to Rao the digestive gland is the main source of enzymes.

Readr-Trevor (1976) has not only studied the normal digestive gland but also studied the infected digestive gland of *Bithynia tentaculata*. He described the digestive gland was composed of two main cell types *viz*. digestive and secretory. He further explained that the digestive cells appear to be concerned with absorption and digestion, whereas the secretory cells produced digestive enzymes. The undifferentiated cells were scattered between them. The pathological effects of the larval digeneas (cercaria heletica) on the digestive gland were investigated at the ultrastructural level. The digestive gland of infected snails appeared to be degenerating.

The ultrastructure of the cells, in the digestive gland of the slug, *Ariolimax reticulatus* was studied by Walkar(1971). On the basis of structural differentiation he identified and described four kinds of the cells *viz*. thin or undifferentiated cells, calcium secreting cells, digestive cells and secretory cells. The calcium secreting cells showed the spherules of calcium salts, whereas the digestive and secretory cell contained green and yellow granules respectively. The excretory cells were distinguished from the digestive cells by having a large central vacuole containing excretory granules. Histochemically these granules were just like the yellow granules of the digestive cells, composed of lipofuscin, suggesting that the digestive cells arising from the excretory cells. The fine structure of the digestive tubules of bivalves was worked out by Owen (1972-a). He pointed out that the digestive cells were replaced by darkly staining cells.Lateron the digestive diverticula of protobranch bivalve, Nucula sulcata was investigated by Owen (1972-b) using electron microscopy. He observed that the peroxisomes were located at the basal region of the epithelial cells. Pal (1972) studied the fine structure of the digestive cells in digestive diverticula of Mya arenaria, and found that these cells were participated in the process of endocytosis and subsequently intracellular digestion. The digestion in filter feeding bivalves was studied by Purchon (1971). He reported that the digestion involves a chronological sequence of events associated with other bodily functions, such as the phases of activity and quiescence of adductor muscle which in turn might be dependent on tidal, diuranal or endogenous rhythms. The process of digestion and absorption in the digestive epithelium were organized in distinct phases throughout the digestive diverticula. Marton (1970) studied the cytological structure and functions of the digestive diverticula of Macoma balthica and correlated with the tidal rhythm. He observed that the absorption in the digestive diverticula of the food material derived from the sea only, when the animal was covered by the tide.

The fine structure of the adult digestive gland of Lymnaea stagnalis was studied by Arni (1974). He reported that the epithelium of the gland exhibited 6 different types of cells, termed as A, B, C, D, E and F cells. He further described the structure and functional significance of these cells. The 'A' cell was undifferentiated and represented the precursors of other cells, whereas the 'B' cell contained the vacuoles of different structure and size, in which the food was digested. The 'C' cell had been called the secretory (or lime cell). The cytoplasm of the 'D' cell contained the excretory granules which were periodically released in the lumen of the gland. The 'E' cell contained much glycogen and osmophilic droplets, which presumably consisted of lipids, whereas 'F' cell possessed large atypical cytosomes and reduced cytoplasm. These cells would be reabsorbing cells or they might be represented an intermediate stage of other cell types.

Morton (1975) studied the seasonal variations in the feeding and digestive cycle of Amphibola crenula . He described that the animal fed actively when on the mud surface, but did not feed after burried in the mud. The cycles were related to the seasons, during the winter the animals were feeding actively at low tide, whereas in the summer they were active in feeding through whole day, except for a short period at high tide. The digestive diverticula complete a cycle of absorption and assimilation every 24 hours.

B) Review of the existing literature on the alimentary tract of the Mollusca :

a) Other studies :

A critical analysis of the existing literature on the alimentary tract of molluscs revealed that much of the work is done on the presence of enzymes and in few cases lipids, proteins and pigments. Enzymes, particularly the lysosomal and digestive enzymes from the molluscan alimentary tract have been studied and are also being investigated by several investigators in Japan, U.K., U.S.A. and Italy. The following is a brief summary of the literature available on enzymes of the molluscan alimentary tract.

i) Enzymes :

Kimura et al. (1966) have studied enzymes from the digestive tract of Helix peliompnala, particularly chitinase and considered the role of this enzyme in chitin formation and decalcification of the chitin. Halton and Owen (1968) reported that the gastric cuticle of the bivalve, Nucalat sulcata was enzymatically active and showed a strong positive reaction for acid phosphatase, whereas it reacted weakly towards non-specific esterase and aminopeptidase. Similar histochemical study was carried out by Eble (1968) on American Oyster and found that two enzymes viz. glycogen phospharylase and branching enzyme were concerned with the glycogen metabolism. These enzymes were detected in the alimentary canal. Bowen (1970) studied the fine structural localization of acid phosphatase in the gut epithelial cells of the slug, Arion ater, wherein the positive enzyme activity was detected in the multivesicular bodies and cytoplasm of the columnar cells of the crop, multivesicular bodies of the intestine and vacuolar localization in the digestive cells of the intestine and digestive gland.

Greenfield (1972) studied the enzymes in the alimentary tract of A. spinigera and observed that the amylases were most active at pH 6.0 - 6.5 . He further pointed out, the digestive enzymes were distributed throughout the intestinal tract, but the amount of the enzymes varies from region to region and was greatest just after feeding.

Patton and Quinn (1973) have studied the digestive lipase activity of the surf clam, Spisula solidissima and compared with that of the hog pancreatic lipase. The pH optima was found to be 8.0 at $20^{o}C$. The lipase activity was incapable of hydrolyzing oleate and palmitate esters of secondary alcohol of glycerol, cholesterol or isopropyl alcohol but was capable of hydrolyzing oleate ester of isobutyl alcohol, a primary alcohol. Kulkarni (1973) investigated on the digestive enzymes of the land slug, Laevicaulis alte . He reported that they were acidic in nature and could act on maltose, lactose, sucrose, raffinose, glycogen and starch but not on cellulose. The amylase of the midgut gland had a pH optima at 5.2 at 35°C. Lipase was detected only in the midgut gland. Mantale (1973) studied in detail the pH range of the alimentary canal and of the digestive enzymes in the land snail, Cryptozona semirugata and reported that the salivary gland amylase, maltase, lactase were strong, whereas glucogenase and protease were weak and digestive diverticula showed a wide range of carbohydrases including a weak cellulase, lipase and protease.

Mather (1973) studied the extracellular enzymes in Ostrea edulis with special reference to carbohydrate metabolism viz. B(1-4) polysaccharide, maltase, α-amylase, dextrin (1-6) glycosidase, α-glycosidase acting on sucroses and B-galactosidase and suggested that these enzymes were released into the lumen of the stomach by the dissolution of the style and by the stomach wall, whereas inulinase or chitinase were not identified. True cellulase activity was demonstrated only in the extract of the digestive diverticula. The midgut was found to be enzymatically active and attributed with a role of continued digestion. Ibrahim et al. (1974) demonstrated highest activity of alkaline phosphatase in the mucous secreting cells but lowest activity in the region where goblet cells were absent in the alimentary tract of snail, Marsia cornuaretis. Moreover, the cuticular or chitinous formation showed no trace of any enzymatic activity.

Carbohydrases in the alimentary canal of the terrestrial slug, Achantina fulica were studied by the paper partition chromatography and α-glucosidase, α-galactosidase, B-galactosidase were recorded from the pharynx, esophagus, stomach, intestine, esophageal gland and midgut gland. Amylase was detected from all the regions of the alimentary tract except the intestine. These enzymes seem to be secreted continuously regardless of feeding stimulus (Chaturvedi et al., 1974). In a similar manner Gunner et al.(1976) studied chitinase B-N-acetyl glucosaminidase in the digestive juice of the snail, Alelix pomatia . Recently Fantin et al. (1982) have studied histomorphology and histochemistry of the digestive apparatus of Murex brandaris and Trunculariopsis trunculus. They found that the digestive enzymes were not produced in the stomach and/or intestine but were produced by the connected gland as well as a specialized epithelial cell type (glandular cell) present in the esophagus and in rectum.

ii) Proteins :

Relatively very little is known about the proteins in the alimentary tract and associated glands of the molluscs. Matus (1971-b) showed that the tubules of the posterior salivary glands in Octopod molluscs consisted of two distinct layers of epithelia viz. type A and type B, the former being predominant. He demonstrated the presence of biogenic monoamines in small proportions in the columner cells, of type A epithelium. Rajyalakshmi-Bhanu et al. (1981-a) reported on the presence of rich quantities of basic proteins, protein bound aminogroups, sulfhydrils and moderate quantities of proteins with tryptophan and tyrosin in the foregut and midgut gland in T. bufo.

iii) Lipids :

Boucaud-Camou (1968) demonstrated the presence of lipid inclusions in the liver of Sepiola atlantica d'orbigny and Sepia officinalis. While studying the histomorphophysiology and histochemistry of the digestive apparatus of Murex brandaris and Trunculariopsis trunculus. Fantin et al. (1982) have also analysed biochemically the polar lipids from the connected gland. Chatterjee and Ghose (1973) reported that the lipid level rises in the digestive gland of two freshwater prosobranchs, Viviparous bengalensis and Acrostoma variable from June to September, which is followed by a gradual depletion.

iv) Pigments :

Boucaud-Camou (1968) demonstrated several inclusions including pigments in the liver of S. atlantica d'orbigny and S. officinalis. Moreover, in S. officinalis the calcarious granulations are actually ferric pigments.

b) Mucosubstances :

A critical evaluation of the existing literature on the mucosubstances of the alimentary tract by Bhide (1979), Mutkekar (1982), Mangalware (1982), Patil (1983), Mandlik (1983) and Deshmukh (1983) revealed that much of the work has been carried out on the vertebrates, particularly the mammals. In comparison very little is known about the alimentary tract mucosubstances of the invertebrates. This stimulated to undertake the present investigation on mucosubstances in the alimentary tract of a marine slug, Oncidium verraculatum. Some idea of the work done on mucosubstances in the alimentary tract of different molluscs can be obtained from the following brief review :

Boucaud Camou (1968) reported on the structure of the digestive organ in Sepiola atlantica d'orbigny and Sepia officinalis. He further reported that the structure of the digestive organ was similar in both species, but their livers were somewhat different. Moreover, the liver in both species contained mucopolysaccharide together with lipid, glycoprotein and pigments. Demian and Michelson (1971) have studied the epithelial mucins of the alimentary tract of a snail, Marisa cornuarietis by employing histochemical tests. They identified four types of mucosubstances in the six different types of cells viz. neutral mucosubstances with moderate to weak reactivity in the fusiform and club-shaped cells, strongly PAS reactive neutral mucosubstances in the ovate cells, PAS unreactive but strongly acidic sulfomucins in the goblet cells and saccular cells and PAS reactive but weakly acidic sulfomucins in the elongated conical cells.

Rahemtulla and Lovtrup (1975) have isolated acid mucopolysaccharides from Anodonta by ion-exchange chromatography. They identified four fractions of acid mucopolysaccharides viz. chondroitin, hyaluronic acid, highly sulfated chondroitin sulfate and heparin. Rajyalakshmi-Bhanu et al. (1981-a) observed two types of cells viz. secretory and absorptive cells in the foregut and midgut glands of a neogastropod mollusc, Thias bufo . These glands were found to be loaded with rich quantities of carbohydrates and moderate quantities of glycoproteins. In a later study Rajyalakshmi Bhanu et al. (1981-b) showed the presence of two types of alveoli (mucous and serous) in the salivary glands of T. bufo. They further demonstrated the presence of acid, sulfated, neutral mucopolysaccharide, sialomucin, hyalomucin and glycoprotein in the salivary glands. Chatterjee and Ghose (1974) studied glycogen levels in the digestive gland of two fresh water prosobranchs, Viviparous bengalensis and Acrostoma variabile. They found seasonal variations in the glycogen content of the digestive gland, the glycogen level being lowest in January which goes upto in the months from January to September followed by depletion from October onwards.

c) Reasons that stimulated the undertaking of the present investigation :

A critical evaluation of the available literature on animal mucosubstances in general and alimentary tract mucosubstances in particular, reveals that there are several avenues open for young scientists working in the field of histochemistry and biochemistry of mucosubstances in the gastropod alimentary tract. The literature on animal mucosubstances significantly shows that :

i) Though the various tissues and organs-system of vertebrates such as connective tissue, cartilage, reproductive tract, kidney, muscles, salivary glands have been elaborately investigated with a view to find out their localization, histochemical and biochemical characterization, isolation and purification of mucosubstances and wherever possible some physiological roles have been attributed to the mucosubstances, comparatively out status of the knowledge on mucosubstances in the gastropod alimentary tract is poor.

ii) Moreover, whatever work has been done on alimentary tract shows that mammals are the focal point for such studies. As compared to the mucosubstances in the mammalian alimentary tract very little is known about these components in the alimentary tract of gastropod.

iii) Whatever work has been reported on the mucosubstances in invertebrate alimentary tract, some investigators have selected one organ of the same animal others have selected altogether a different organs of different animals. Hence, as the literature on mucosubstances in the alimentary tract stands today, there is very scanty literature on detailed investigation on mucosubstances in all the organs of the alimentary tract of one and the same animal.

The above critical evaluation of the existing literature on alimentary tract mucosubstances makes it very clear that a detailed investigation of mucosubstances in gastropod alimentary tract is essential to augment their nature and role played in the physiology of the alimentary tract. It is with this view, that an extensive research project has been undertaken in this laboratory to study the mucosubstances in various organs systems in different invertebrates. When the entire research project will be completed, most of the lacunae in our present day understanding of invertebrate alimentary tract mucosubstances will be satisfactorily removed. The work included in the present thesis forms a part of this extensive resèarch scheme and concern with mucosubstances, their histochemical characterization and distribution in various organs of the alimentary tract of O. verraculatum.

C) Plan of the present investigation :

Keeping in view the aforementioned points and meagre amount of work done on the alimentary tract mucosubstances in Ophisthobranch, it was decided to work out the histology of the various organs and distribution and characterization of mucosubstances in the alimentary tract of O. verraculatum.

i) <u>Choice of the animal</u> :

While selecting the animal care was taken to select such an Ophisthobranch wherein no work has been reported on mucosubstances in any part of the alimentary tract. Secondly, the animals should be available in required number throughout the course of the investigation. Therefore, the ophisthobranch, *O. verraculatum* was found most suitable for the present investigation.

ii) <u>Choice of the techniques</u> :

Since the present investigation aims at a detailed study of the nature of mucosubstances present in different cellular sites in alimentary tract from buccal mass to intestine of *O. verraculatum*, characterise them histochemically recent and well established histochemical techniques were employed. The histochemical techniques have an advantage over biochemical techniques in the fact that though the latter techniques give reliable data on the quantitative presence, they are not of much use in illustrating the tissue and cellular localization. From this point of view, the histochemical techniques are better. In the present investigation, the staining timings were kept constant throughout the work, and differences, if any, in the intensity of staining were taken as reflections a differences in the concentrations of the different types of mucosubstances.

iii) Critical evaluation of the observations :

It was decided to analyze critically the results obtained in the present investigation on alimentary tract of *O. verraculatum* in relation to :

a) Histology of the various organs such as buccal mass, esophagus, stomach and intestine.

b) Histochemical characterization of the mucosubstances in different layers from mucosa to serosa in different organs of the alimentary tract.

c) The distribution of mucosubstances in different layers from mucosa to serosa in different organs of the alimentary tract.

d) To compare the results obtained in the present investigation and the existing literature on other molluscan so as to find out similarities or difference, if any.

e) To find out cellular specializations, if any, in a given organ of the alimentary tract.

f) To project idea about the functional significance of mucosubstances in the various organs of the alimentary tract based on circumstantial evidences.

D) Presentation of the Thesis :

It was decided to divide the present thesis into four chapters, the first chapter being introduction, it gives a brief review of the existing literature on mucosubstances in various organs of the alimentary tract of mollusc, reasons that stimulated to undertake the present investigation and the plan of the proposed research work. The second chapter deals with the material and methods employed in the present investigation. Detailed histological and histochemical observations on buccal mass to intestine of the O.verraculatum are listed in chapter III. The chapter four is devoted to the discussion on results obtained in the present investigation and existing literature. The last chapter will be followed by summary and concluding remarks. A complete bibliography of the literature cited in the various chapters of the present thesis is given at the end of the thesis.