CHAPTER - ONE

INTRODUCTION

Review of the literature, reasons for undertaking the present investigation and plan of proposed work.

The phylum Annelida comprises the segmented worms and includes the familier earthworms and leeches, plus a great number of marine and freshwater species of polychaete worms. The phylum contains over 8700 described species, which are placed into three classes: Polychaeta, Oligochaeta and Hirudinea. The ancestral annelids were probably marine animals burrowing in the bottom of the sand and mud of shallow costal waters. The class Polychaeta contains most of the living marine species. The class Oligochaeta, which includes the freshwater annelids and terrestrial earthworms, may have stemmed from some early polychaetes, but more likely evolved independently from the ancestral annelids. The class Hirudinea, the leeches clearly arose from some stock of freshwater oligochaetes (Clark, 1969).

In general, members of the phylum Annelida are vermiform metameric animals. The metamerism is probably evolved as an adaptation for peristaltic burrowing in soft substrata. The primary metameric structures are the body wall muscles and the coelomic compartments. The nervous, circulatory and excretory systems are metameric. The gut is typically a straight tube extending through the body between anterior mouth and posterior anus. The gut is located in the coelom by longitudinal mesenteries and by the septa, through which gut penetrates Digestion is extracellular. The anatomical organization of the digestive tract of annelids includes regions of foregut, midgut, and hindgut, derived from stomodeal ectoderm, endoderm and proctodeal endoderm respectively. Regional modifications in the structure and function occur both within and between various classes reflecting wide variations in mode of life, uptake of food and dietary habits.

Before the turn of this century the annelids were studied for their morphology, taxonomy, systematic position and evolution. Since then however, the striking and growing awareness of their importance to the ecology and bioeconomics stimulated scholorly interest in the group so that today our knowledge of annelida has grown to sizable proportions. At present a voluminous data have accumulated on their evolution, taxonomy, morphology, anatomy, ecology, bioeconomics etc. Research on physiology of annelida may have any of the three objectives. The first is detailed analysis of fundamental physiological mechanism. The second is to gain an understanding of the way in which animal meets the physiological requirements of living in a particular environment and performing certain functions. The third is to set the physiological mechanism of different animals in an evolutionary context. In recent years there has been markedly less enthusiasm on the part of comparative physiologists for making comparison between related or unrelated animals than was formerly the case, and even less fashionable is the evolutionary approach. The knowledge of annelid biochemistry and physiology was to overwhelming extent derived from only a handful of species: a few nereids and the earthworm. Comparatively less attention was paid to the histological architecture of the alimentary canal in annelids, and so is true for the distribution of mucosubstances and other metabolites in different physiological regions of the alimentary canal. Detailed account of anotomy, physiology and development of Indian earthworm, Pheretima posthuma was given, for the first time by Bahl (1926). He reported that the alimentary canal of P. posthuma is a straight tube running along the entire body length. The month and anus constitute its anterior and posterior openings respectively. It is functionally regionated into various parts which are buccal chamber, pharynx, oesophagus, gizzard, stomach and intestine. Further, Bahl (1926) described the histology of gut wall in P. posthuma. The wall of the alimentary canal consists of four layers in succession. Peritonium is the outermost layer consisting of tall and narrow cells. On the intestine and sometimes on the stomach. some of these cells contain yellow reffractile granules. These cells are yellow or chloragogen cells. Under the peritonium lies a layer of muscles. Muscles include an outer layer of longitudinal muscle and an inner layer of circular muscle fibres. They are well developed in pharynx and oesophagus but ill-defined in the intestine. In gizzard circular muscles are much developed, while longitudinal muscles are absent. The muscles are unstripped or involuntary. The interior of gut wall has lining of epithelial cells which is internally thrown into folds in oesophagus, stomach and intestine. It consists of a single row of columner cells which become ciliated on the roof of pharynx, glandular in stomach and absorptive and glandular in the intenstine. Internal epithelial lining is lined by thin lining of cuticle in buccal cavity and in the gizzard as a thick lining Bhatia (1941) reported on anatomy and physiology of Indian cattle leech, *Hirudinaria granulosa*. Morphologically, the alimentary canal of leech is a complete straight tube extending from mouth to anus. It is differentiated into buccal cavity, pharynx, oesophagus, crop stomach, intestine and rectum. Histologically, the alimentary canal consists of a layer of columner epithelium separated by a basement membrane from an outer layer of connective tissue. The columner epithelium is lined by cuticle in the fore and hind guts, it is also ciliated in the hind gut and contains scattered goblet cells in the crop. The connective tissue layer contains haemocoelomic capillaries and circular muscle fibres; in the preoral chamber it contains circular as well as longitudinal muscle fibres, while in the pharynx it contains circular, longitudinal and radial fibres. In the present investigation the earthworm, *P. posthuma* and cattle leech *H. granulosa* were used since these are widely distributed in this region and the histochemistry of the alimentary canal in these two annelids has not been worked out.

I. REVIEW OF THE LITERATURE ON THE ALIMENTARY CANAL OF ANNELIDS :

The last two or three decades have witnessed an appreciable upsurge in our understanding of physiology of digestion in annelids, their dietary habits, morphological variations in the alimentary canal region, enzymes secreted by different regions and their role in digestion of food etc. In this regard the most of the annelids have been studied mode of feeding and gut structures and biochemistry of digestion. The following brief review will give an idea about the gut structure and feeding in oligochaetes and Leeches.

A. GUT STRUCTURE AND FEEDING IN OLIGOCHAETES :

1. A. Anatomy of the Digestive Tract :

Oligochaetes are considered omnivorous but feeding habits are diverse. Many terrestrial forms feed primarily on leaf and other plant material, but also consume large quantities of soil and associated organisms. Some aquatic forms are active predators, where as other live primarily on detritus (Van Gansen, 1962; Dales, 1963; Jeuniaux, 1969; Edwards and Lofty, 1972).

The prostomium which is small and occassionally may be absent (Stephenson, 1930) is greatly considered to have tactile function, (cook, 1972). In Aeolosoma bengalense the ciliated ventral portion functions in feeding and locomotion (Postwald, 1971). In lumbricids (Arthur, 1965) and Pheretima (Bahl, 1926), it may act as downward scoop bringing food into the preoral canal.

There is considerable variation in the anatomical features of the alimentary canal in the smaller aquatic forms (Microdrilli) and the larger, primarily terrestrial worms (Megadrilli) (stephenson, 1930; Avel, 1959; Van Gansen, 1962; Cook, 1972).

1.a. Structure of the foregut :

In general, the mouth is located beneath the Prostomium, opens into a short buccal cavity, which is lined with the cuticle continuous with that of the body wall. The buccal cavity may have diverticula and has an associated musculature which permits protrusion of the ectoderm, which contains sensory structures (Semal-Van Gansen, 1957; Van Gansen, 1962; Arthur, 1965; Palka and Spaul, 1970).

Like the buccal cavity, the pharynx has diverticula, but the sensory element is reduced. In lumbricus, a large dorsal diverticulum may show secondary evaginations. The pharyngeal epithelium has a cuticle, and ciliated columnar cells are espeically prominent in the dorsal region. A series of radiating muscle bands act as dilators and retractors of the pharynx, (stephenson, 1930). Protractor and retractcr muscles, attached to the pharynx from the dorsal body wall, have been observed in some naidids and lumbriculids (Cook, 1972).

Associated with the dorsal and lateral wall of the pharynx are present the groups of the glandular (chromophil) cells. These may be located in other areas such as the oesophagus, bodywall, septa or blood vessels, especially in microdriles (Avel, 1959). The ultrastructure of the paired pharyngeal glands, located lateral to the anterior pharynx in the enchytraeid, Enchytraeus albidus, has been described by Reger (1967). Elongate cytoplasmic processes extend from individual gland cell to the pharyngeal lumen and serve to transport secretory product_ especially mucons. A similar morphology has been described for cells in the pharyngeal glands of the lumbricid, Eisenia foetida (Chapron, 1970). In the megascolecid, P. hawayana, Cidre (1972) has observed the elongate processes in those cells forming circular ring behind the gizzard. The pharyngeal glands of earthworm are also mainly mucus producers; the mucus lubricating the food (Van-Gansen, 1957).

The oesophagus opens from the pharynx as a narrow tube with various diverticula and 'Calciferous' glands and may be modified into a dilated thinwalled crop and thick-walled muscular gizzard. Chloragogen cells, derived from the peritoneum, begin to appear in the oesophageal area, especially in microdriles, where the separation of oesophagus and intestine is often difficult to distinguish. The crop is most prominent in megadriles. The gizzard is present in all families of megadriles and may be multiple. A post pharyngeal stomach, which may or may not be a dilation, but is histologically distinct from the rest of the gut (Stephenson 1930).

Histological studies by Palka and Spaul (1970), in the enchytracid, Lumbricillus lineatus, showed that the oesophagus is lined with cuboidal nonciliated epithelium and there is no crop or gizzard. In P. hawayana, the gizzard is preceded by a primary oesophaugs of simple columner epithelum and followed by a secondary oesophagus (stomach) with a striated border of columner epithelium (Cidre, 1972). In E.foetida, the oesophagus is lined by a simple cuticulated epithelium and gland cells are rare (Burke, 1974). The crop and gizzard possess well developed brush border and epithelial secretions include mucus and proteins. The epithelium of the gizzard produces a thick elastin cuticle (Van Gansen, 1962).

The pharyngeal bulb in P.posthuma, has aggregates of pharyngeal gland cells (Bahl, 1950). The gland cells are large in size but vary in shape; their function is to produce mucus and a proteolytic enzyme (Keilin, 1920). The saliva is conducted into the lumen of the pharynx by means of salivary channels which traverse the musculo-vascular tissue and on reaching the pharyngeal epithelium, divide into a great many ductules which penetrate the epithelium near the free surface (Bahl, 1950). The internal lining of the columner epithelium is covered with an internal cuticle . The glandular structures causing external swelling on the stomach are the calciferous glands (Stephenson and Prashad, 1919) and secrete a calcareous fluid. In Pheretima there are no calciferous glands but the transverse folds of internal epithelium of the stomach may be regarded as the simplest condition of calciferous glands (Bahl, 1926).

1.b. Structure of the Intestine :

In general, the intestine of microdriles is a simple cylindrical tube without typhlosole and contains an epithelium of ciliated columanar cells, a thin muscular layer and a layer of chloragogen cells (Cook, 1972). There is a small typhlosole along the major part of the intestine. Large glandular cells are scattered among the prominent non-glandular ciliated columnar cells, especially in the mid-portion of the intestine (Palka and Spaul 1970).

Four well defined regions have been described for the intestine of _E. foetida_ (Van Gansen 1962). The contractile anterior intestine (Segment 20-25) has dilations and its epithelium bears a brush border and secretes mucus and proteins. The epithelium of the middle intestinal region (Segments 26-44) is ciliated and the typhlosole, which arises more anteriorly, is very pronounced, ciliated and contains glandular cells. The posterior intestine (Segments 45-97), characterized by the presence of a peritrophic membrane, is ciliated and the volume of the typhlosole decreases. The cuticulated epithelium of the proctodaeum is continuous with that of the ectoderm.

The intestine of Lumbricus terretris is also divided into four regions. (Arthur, 1963). In P. hawayana (Cidre, 1972) the typhlosolar intestine and associated caecae have epithelium of ciliated cells alternating with club shaped glandular cells which are abundent in the typhlosole. The posterior or terminal intestine contains prismatic cells with tonofibrils.

Intestine in P. posthuma can be distinguished into three regions, pre-typhlosolar, typhlosolar and post-typhlosolar region (Bahl, 1926). Wall of the pre-typhlosolar region is folded internally to form minute processes, the villi, and is highly vascular. This region bear intestinal caecae. Typhlosolar intestine is characterised by the presence of a highly glandular and vascular longitudinal ridge, arising as a median ingrowth of the dorsal aspect of the intestinal cavity, called the typhlosole. Post-typhlosolar intestine or rectum bear internally longitudinal folds. Internal epithelium of the intestine consists of a single row of columner cells which become absorptive and glandular in the intestine (Bahl, 1950).

2. TRANSPORT OF FOOD THROUGH THE ALIMENTARY CANAL :

In the enchytraeid, L. lineatus, anterior segments may be pushed over the food which is drawn into the buccal cavity by expansion of the pharynx and/or the pharyngeal plate which may be protruded as a cup-like structure embracing the food to draw it into the pharynx. Contraction and expansion of the gut and body will draw the food through the pharynx into the oesophagus. Food usually appears in the intestine within three to four minutes. Feeding ceases when the anterior portion is filled, but starts again when the contents are reduced or passed on, and faecal matter appears in the last segments. The passage of food in the intestine is aided by the action of the predominent ciliated cells of the epithelium (Palka and Spaul, 1970). In the stomach food remains for two to seven minutes, in the anterior part of the intestine for 120-280 minutes and travels through the posterior part of the intestine in 50 minutes (Szarski, 1936).

In lumbricids, the food adheres to mucus extruded with the buccal epithelium. The pharynx exerts a further suction and the food enters the pharynx, which also functions as a force pump reinforcing oesophageal peristalsis and propelling food as far as the gizzard. Ciliary action may augment muscular contraction in the anterior oesophagus. The crop contracts more frequently than the gizzard which acts as a force pump for transport of materials in the intestine. The presence of solid material appears to be the stimulus for contraction in both regions, and there is no difference in the physical state of their contents (Arthur, 1965). The fold of the crop regulate the rate of movement of material into the gizzard, prevent regurgitation, and may assist in the mixing of food (Arthur, 1963). A sphincter of circular muscles controls the passage of food from gizzard to tubular intestine. The sac like dilations of the anterior intestine show rhythmic expansion and contraction. Intestinal ciliary mechanism do not appear to act in propelling food along the tract. Body wall movements during locomotion influence the unfolding and folding of the gut (Arthur, 1965).

3. CONTROL OF GUT MOVEMENT :

The gut shows peristaltic activity (Gardy, 1957) and appears to be under the nervous and hormonal control (Wu, 1939; Millott, 1943 a, 1943 b). Some movement of the gut contents is due to an indirect action of the body wall, but all parts of the gut, however thinly muscled, are capable of constricting movements which may travel in either directions. Wu (1939) reported that the earthworm gut is innervated by antogonistic nerves which are andregenic and cholinergic respectively, Millott (1943 a, b) showed that this was the case in L. terretris. The system of nerves controlling the tonus of the gut, is composed of cholinergic augmentors fired from median and posterior segmental nerves through the body wall and entering the gut ventro-laterally across each septum, and andrenergic inhibitors also fired through the anterior segmental nerves and entering dorso-laterally. There is no knowledge of whether there is similar mechanism in other annelids, but it is clear that the movements of the gut are delicately controlled, and that the contents are mixed and moved in a variety of ways.

4. BIOCHEMISTRY OF DIGESTION :

4.a. Digestive Enzymes :

The investigation of the complement of digestive enzymes has been limited primarily. Protease, lipase, amylase, lichenase, cellulose and chitinase activities have been described and each enzyme has a distinctive pH optimum (see Laverack, 1963). The biochemical properties of these and the other digestive enzymes have been reviwed by Jeuniaux (1969).

Observations on the microdriles have been limited. Histochemical observations by Szarski (1936) suggest that protein, fat and starch digestion occure principally in the fore and mid-intestine in naidids. A variety of carbohydrases have been detected in whole animal extracts in the enchytraeids, Cognettia sphagnetorum and Fredericia hegemon, and B-glucosidase and B-galactosidase are found in the intestinal walls of Mesenchytraeus glandulosus (Nielsen, 1962). Animal extracts of the naidid, Paranais littoralis, hydrolyze 6 (amylase, Glycogen, laminarin, chitin, maltose and cellobiose) of 29 carbohydrates tested (Kristensen, 1972). Trypsin, with properties comparable to the enzyme isolated from L. terrestris, has been isolated from whole animal extracts of the tubificid, Branchiura sowerbyi.

4.b. Intestinal and Epidermal Absorption :

In E. foetida the posterior intestine with the characteristic peritrophic membrane appears to be a major zone of absorption (Van Gansen, 1962). In Lumbricus costaneus, alkaline phosphatase activity is high the typhlosole (segments 65-70) of the posterior intestine (Haase, 1969). Tissue homogenates of segments ¯20 - anus of P. hawayana have a higher activity than segments 1-4 or 5-19 (De Jorge and Sawaya 1967). An intestinal reaction has been localized at the luminal border of the typhlosolar cells and villi of the intestinal epithelium of P. posthuma, and the reaction gradually fades in the posterior intestine (Vyas and Dev 1972).

B. GUT STRUCTURES AND FEEDING IN LEECHES :

1.a. Anterior Part of the Gut :

Most leeches are blood-sucking species but some are predators. The anterior part of the digestive tract shows a wide variety of anatomical structures in harmony with the various procedures involved in the uptake of food. The classification of leeches is based on this anatomical diversification. The forgut includes the mouth (generally situated in the anterior sucker), buccal cavity, pharynx and oesophagus (Harant and Grasse, 1959).

In *Glossiphonia complanata* (Damas, 1962, 1969), the buccal cavity is transformed into an eversible sheath which possesses powerful longitudinal muscles. Between this sheath and the oesophagus, the long muscular pharynx has evolved into a protractile proboscis. Epithelia of the proboscidian sheath and proboscis, rich in alkaline phosphatases, secrete the collagen cuticle which is thicker than the body cuticle and continuous with it. The salivary glands are holocrine, unicellular glands which possess individual excretory ducts grouped into two bundles lateral to the oesophagus. Mucous glands open into the cavity of the proboscis sheath, whereas proteinaceous glands discharge at every level of the proboscis lumen. They also contain alkaline phosphatases.

Most of the Gnathobdellae are blood sucking animals (e.g. *Hirudo medicinalis*, *Macrobdella* and *Hirudinaria*), whereas *Haemopis* is a predator which swallows small invertebrates.

In *H. medicinalis* the wide mouth leads directly to the pharynx which is provided with. three jaws, one median-dorsal and two ventro-lateral ones. These jaws consists of muscular swelling of the pharyngeal wall lined with collagen cuticle (Damas, 1972).

The unicellular salivary glands are dispersed in the connective tissue from the base of the jaws to the middle of the crop. Their individual secretory ducts are arranged in three bundles which emerge in the corresponding jaw. With histochemical techniques (Damas, 1974) it is possible to distinguish mucous glands which secrete acid mucopolysaccharides from serous glands which synthetize histamine, hyaluronidase and proteases. In H. granulosa some salivary glands show adenosine triphosphatase activity and their secretion is emitted through ductules opening around the teeth (Dev and Mishra, 1971).

In the Gnathobdellae the pharynx is provided with a powerful muscle coat consisting of circular, longitudinal and radial fibres. In the bloodsucking leech, H. medicinalis, the radial muscles are particularly developed, while in the carnivorous predator Haemopis the circular muscles predominate (Hammersen, 1963). The short oesophagus may be bent and represents only a transitory passage between pharynx and crop.

In, H. granulosa the foregut is highly specialized for blood-sucking mode of feeding. It consists of pre-oral chamber, buccal cavity and pharynx. The mouth leads into a very short chamber, the buccal cavity. The mucous membrane of buccal cavity bears crescentic jaws (Bhatia, 1941). The jaws which are three in number, are monostichodont and resemble a semicircular saw. The buccal cavity leads through a very narrow aperture into the pharynx. The wall of the pharynx is highly muscular.

The salivary glands are large masses of pyriform unicellular glands. Each salivary gland cell is a gland itself and is produced into a long ductule, all ductules running forward in thick bundles along the wall of the pharynx to enter the three jaws. The active substance in the saliva preventing coagulation of blood is hirudin or anticoagulin (Bhatia , 1941). Further, Dev and Mishra (1971) showed adinosine triphosphate activity in some salivary glands in H. granulosa.

Histologically, the wall of fore gut has a inner layer of columnar epithelial cells surrounded by a layer of connective tissue, containing muscle fibres (Bhatia, 1941). The connective tissue in the pre-oral chamber contains circular and longitudinal muscle fibres. Whereas in pharynx it contains radial muscle in addition to the circular and longitudinal muscle fibres (Bhatia, 1941).

1.b. Middle and Posterior Parts of the Gut. :

The crop is the longest segment of the digestive tract and often possesses numerous pairs of blind diverticula or caeca, the arrangement of which has been well described (Herter, 1968). Generally the last pair is longer and can reach the posterior end of the body.

The structure of the crop in Hirudo has been examined in normally feeding and fasted animals under the optical (Diwany, 1925) and electron microscope (Hammerson and Pokahr, 1972 a). The unistratified epithelium is lined with a striated border composed of numerous microvilli and its cytoplasm contains many apical mitochondria and abundant fatty inclusions. During prolonged fasting, the fats are reabsorbed and represents a reserve of energy.

There is often a sphincter between crop and intestine. In numerous leeches the intestine is a straight tube and narrower than the crop. The intestinal epithelium has the same type of structure as that of the crop. In H. medicinalis the cells also contain fat and glycogen (Diwany, 1925) and ultrastructural observations (Hammersen and Pokahr, 1972 b) show a thick apical covering of microvilli, a gradient in the distribution of mitochondria and important folds of the basal membrane.

The intestine is separated from the rectum by a sphinctor. The rectum is an S-shaped tube which often widens into a ciliated bladder. The anus lies dorsally, just before the posterior sucker.

The crop in H. granulosa, comprises the largest portion of the alimentary canal and occupies about two-third of the visceral space (Bhatia, 1941). The crop is divided by narrow constrictions into a series of ten to elevan chambers. From each chamber arise a pair of lateral backwardly directed diverticula or caeca. The stomach is a small rounded chamber with its internal wall much folded. The intestine is a narrow tube. Inner wall of the intestine has longitudinal and transverse folds. Thin walled rectum opens to the exterior by anus.

Histological studies on H. granulosa by Bhatia (1941) clearly indicate that the wall of the gut consists of a layer of columnar epithelial cells separated by a basement membrane from an outer layer of connective tissue. The columnar epithelium is lined by cuticle in the fore and hind guts, it is also ciliated in the hind gut and contains scattered goblet cells in the crop. The connective haemocoelomic capillaries and circular muscle fibres. In pre-oral chamber the connective tissue contains circular as well as longitudinal muscle fibres, while in pharynx it contains circular, longitudinal and radial fibres.

Besides these preliminary observations on histology, practically nothing is known about the other cellular details in the epithelium in different regions of the alimentary canal. Further, existing data have clearly shown that no work has been carried out on the histochemistry and biochemistry of different metabolites, enzymes etc. in Indian cattle leech, H. granulosa and other leeches.

2. TRANSPORT OF FOOD THROUGH THE ALIMENTARY CANAL :

The uptake and transport of food through the alimentary canal are performed in different ways in haematophagous and carnivorous leeches.

Though most of the haematophagous leeches find their host accidently, it seems the some are able to detect prey by means of their sensory system. Aquatic leeches such as Hemiclepis marginata and Theromizon tessulatum are alarmed by the movements of water or shadows respectively, as generated by a potential host (Herter, 1968).

The Rhynchobdellae, such as Glossophonia complanata the evaginated proboscis, made turgid by the dilation of the blood sinus and by muscular contraction, is used as a stylet to burst into the tissues of the host. The mucus discharged by the salivary glands, eases these movements and the action of muscle of proboscis and pharynx permits the sucking of the body fluids (Damas, 1962)

The Gnathobdellae, such as H. granulosa (Bhatia, 1941) and H. medicinalis (Mann, 1962), pierce the tegument of their victims with their denticulated jaws, which move as minute saws (Herter, 1936, 1968) and produce Y-shaped wound. In the meantime the secretions of the salivary glands are poured into the wound through the canals of the teeth (Damas, 1972, 1974). Biochemically, different chemical, substances have been identified from the homogenates of the anterior region of the body and attributed to the salivary glands. Hyaluronidase as pointed out by claude (1937) and Hahn (1945), has been characterized by Linker et. al. (1957) and Linker et. al., (1960) as a B-endoglucuronidase. The anticoagulant, hirudin, characterized as an antithromboxinase (Lenggenhager, 1936) originates from the hydrolysis of the protein (Yanagisawa and Yokoi, 1938). More recently two forms, α and β hirudin, have been isolated (Jutisz, 1963). The histamine like substance, discharged into the wound, leads to a dilation of the neighbouring blood capillaries (Lindemann, 1939). Both hyaluronidase and histamine have been localized histochemically in the serous salivary gland (Damas, 1974). The blood is sucked by means of the rhythmic contractions of the pharynx. The sucked blood, prevented from clotting and putrefaction, is stored in the large crop diverticula, where it first thickens by removal of water (Worth, 1951).

3. CONTROL OF GUT MOVEMENT :

In leeches digestion may take up to 200 days and is a very slow process. Gut movements are restricted only to sucking of blood. The sucking response is the result of chemical stimulation (Galun and Kindler, 1966, 1968). Thus, H. medicinalis can be fed through an artificial membrane where blood is replaced by a solution of glucose in 0.15 Mol. l^{-1} NaCl; but KCl abolishes the response. The length of the sucking period depends on the solution and is the result of chemoreception at the level of the anterior sucker or in the buccal cavity.

4. BIOCHEMISTRY OF DIGESTION IN LEECHES :

4.a Digestive Enzymes :

Previous studies on proteolytic digestive enzymes are restricted primarily to the sanguivore Hirudo medainalis, and the carnivore Haemopis sanguisuga. Diwany, (1925) found that starved H. medicinalis could not digest milk, egg protein or peptones injected aseptically into the gut. Graetz and Autrum (1935) could not demonstrate proteolytic activity in gut wall extracts of this leech.

More recently, Jennings and Van der Lande (1967) conducted a histochemical investigation on nine species of leeches. Only the salivary glands of P. geometra demonstrated any endopeptidases. However, Damas (1974) has used the gelatin film technique to demonstrate proteinase activity in the salivary gland of H. medicinalis.

Exopeptidase activity (leucyl-B-napthylamide) was observed in the intestinal gastrodermis of all species examined and often in their intestinal lumen (Jennings and Van der Lande, 1967). The intestinal gastrodermis appeared to be the source of the extracellular activity as neither crop gastrodermis nor crop content showed any trace of activity at any time. They suggested that the broad specificity of the naphthylamide substrate is indicative of the presence of several exopeptidases which may act in the absence of endopeptidases by slowly degrading protein chains by progressive removal of terminal units. The leucyl-B-napthylamide reaction was studied in greater detail in E. octoculata and no conclusions could be made about the nature and function of the enzymes involved in the histochemical reaction (Van der Lande, 1972).

Autrum and Graetz (1934) found lipase activity in the intestinal wall and lumen of H. medicinalis and H. sanguisuga. After eating of lipase (pH 8.2 to pH 8.4) was secreted into the crop of H. sanguisuga but not H. medicinalis. The gut gut wall of H. medicinalis may secrete a weak lipase into the lumen (Graetz and Autrum, 1935). Lipases are distributed in the crop and intestine of G. complanata (Damas, 1962).

Besides the hyaluronidase activity of and carbohydrate activities have not been demonstrated in the gut of leeches.

4.b Intestinal Absorption :

Alkaline phosphatase activity has been observed in the apical cytoplasm and striated border of the wall of the crop (especially in the lateral diverticula) and intestine of G. complanata (Damas, 1962, 1965) as well as in the crop and intestinal gastrodermis of all species examined by Jennings and Van der Lande (1967). The results suggests an absorptive role for these areas of the digestive tract. The intracellular distribution of phosphatases in the alimentary canal of Poecilobdella granulosa suggests that the intestine is more involved in absorption than the stomach (Mishra and Dev, 1975).

5. SYMBIOTIC RELATIONSHIP :

The close association of bacteria with the alimentary system of leeches and the limited success in demonstrating a complete complement of endogenous digestive enzymes have led to implication of extensive bacterial involvement in the digestive process.

Busing (1952) identified a single species of bacterium, Pseudomonas hirudinis, in the gut lumen of H. medicinalis and attributed a primary role in digetion to the symbiont (Busing, Doll and Freytag, 1953).

Jennings and Van der Lande (1967) examined the hydrolytic abilities (Protease, lipase, amylase, hemolysin, lecithinase) of mass cultures of bacteria isolated from eight species of sanguivorous and predaceous leeches.

Assessment of the degree of bacterial involvement in the digestive processes of leeches will require a comprehensive series of systematic bacteriological investigation.

II. CRITICAL ANALYSIS OF THE LITERATURE AND 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 alimentary tract of annelids. The literature on annelids significantly shows that :

1. Though the various tissues and organ-systems such as connective tissue, body wall, blood vascular system, nephridia, sense organs, nervous system, reproductive system etc. have been elaborately investigated with the view to find out morphology, histology, histochemical and

biochemical characterization of various metabolites and wherever possible some physiological roles have been attributed to the organ - systems, but comparatively less attention has been paid to the histochemical localization of mucosubstances in the alimentary canal of annelids.

2. At a comparative level morphology of the alimentary canal has been studied in different annelids belonging to different classes such as Polychaeta, Oligochaeta and Hirudinea.

3. The alimentary canal of earthworm and leech has been investigated in detail for its morphology and very little is know about its histology. At present some histological information is available on earthworm (Bahl, 1926) and leech (Bhatia, 1941).

4. Whatever work reported on mucosubstances in the annelids has been focused around mucus secreting structures like slime glands, specialized mucus glands and particularly in the bodywall and practically no attention has been paid on the alimentary tract mucosubstances and their role in physiology of digestion.

5. In some cases only PAS reactivity or AB reactivity of a given cell type in intestinal mucosa or the gland cell or

chloragogen cell has been reported but the mucosubstances have not been further investigated. Recently (Mill, 1978) has rightly pointed out that there is very little work published on the mucins in different tissues of the annelids.

The above critical evaluation of the existing literature on the alimentary canal mucosubstances makes it clear that a detailed investigation of mucosubstances in the alimentary canal of annelids is essential to augment their nature and role played in the physiology of the alimentary canal and also to augment the understanding of invertebrate animal mucosubstances in general. It is with this view, therefore, that this research project has been undertaken in this laboratory. Since very little information is available on the alimentary canal in annelids, particularly at histological and histochemical levels, a detailed study of the alimentary canal in earthworm and leech was thought to give further information.

Thus the present study aims :

1. To find out morphological subdivisions of the alimentary canal and associated structures in earthworm, P. posthuma and Indian cattle leech, H. granulosa.

2. To find out histology of different organs in the alimentary canal of these two annelids.

3. To find out localization of mucosubstances in different layers of the gut wall.

4. To study histochemical characterization and distribution of mucosubstances in the various organs of the alimentary tract of these two annelids.

5. To suggest some functional significance to the mucosubstances in the physiology of digestion in annelids.

III. PLAN OF THE PRESENT INVESTIGATION :

Keeping in view the aforementioned points the meagre amount of work done on alimentary canal mucosubstance in annelids, it was decided to work out the histology of the various organs and distribution and characterization of mucosubstances in the alimentary canal cf the earthworm, P. posthuma and Indian cattle leech, H. granulosa.

A. Choice of the Animal :

While selecting the animals care was taken to select two annelids wherein no work has been carried out on mucosubstances in any organ of the alimentary canal. Secondly, the animals should be available in required number through out the course of the investigation. Therefore, P. posthuma and H. granulosa were found most suitable for the present investigation.

B. Choice of the Techniques :

Histology of various regions of the alimentary canal in the two annelids was studied in Haematoxylene - Eosin (H-E) stained preparation Histochemical staining techniques were employed for the localization of mucosubstances. To characterise mucosubstances histochemically recent and well established histochemical techniques were employed. In the present investigation, the staining timings were kept constant through out the work, and differences, if any, in the intensity were taken as reflections of differences in the concentrations of the different type of mucosubstances.

C. Critical Evalution of the Observations :

It was decided to analyse critically the results obtained in the present investigation on the alimentary tract of P. posthuma and H. granulosa in relation to :

1. Histology of the various organs such as buccal chamber, pharynx, oesophagus, gizzard, stomach and intestine in P. posthuma.

2. Histology of the various organs such as oral sucker, pharynx, crop, stomach, intestine and rectum in H. granulosa.

3. Histochemical characterization of the mucosubstances in different layers in different organs of the alimentary canal of two annelids.

4. The distribution of mucosubstances in different layers in different organs of the alimentary canal.

5. To compare the results obtained in the present investigation and the existing literature on other annelids, if any, to find out similiarities or differences.

6. To find out cellular specializations, if any, in a given organ of the alimentary canal.

7. To project idea about the functional significance of mucosubstances in the various organs of the alimentary canal based on circumstantial evidences.

IV. PRESENTATION OF THE DISSERTATION :

It was decided to divide the present dissertation into four chapter, the first chapter being on the introduction gives a brief review of the existing literature on the morphology, histology, physiology of the various organs in the alimentary canal of the annelids and histochemistry of mucosubstances, reasons that stimulated to undertake the present investigation and the plan of the proposed work. The second chapter deals with the material and methods employed in the present investigation. Detailed histological and histochemical observations on various organs in the alimentary canal are listed in Chapter-III. The last chapter is devoted to the discussion on the results obtained in the present investigation and the 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 dissertation will be given at the end of the dissertation.