CHAPTER - ONE

INTRODUCTION
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

The last two to three decades have witnessed an appreciable upsurge in our understanding of mucosubstances, their chemical nature, identification of individual mucosubstance and functional significance of mucosubstances in relation to life in general. A critical analysis of the existing literature on mucosubstances (Nalavade, 1975; Pawar, 1976; Vibhute,1981; Fartade, 1981) shows that these have been studied at very detailed levels for connective tissue and epithelia. In addition the mucosubstances have also been studied in respiratory tract, nervous system,kidney, major salivary glands,lingual glands, muscles, gonads and associated glands in the reproductive tract and neoplasma. Although, mucosubstances have been studied in the gastrointestinal tract, the laboratory animals such as rat, rabbit,guinea pig etc. are the focal points for such studies. In this regard the various organs of the gastrointestinal tract of mammals belonging to the lower orders and submammalian vertebrates have not received attention as they deserve. Recently Loo and Wong (1975) have pointed out that "the survey of the literature reveals that little has been published on mucins of the gastrointestinal tract in Anurans." This prompted to undertake the present investigation on mucosubstances in the gastrointestinal tract of the locally available frog,Euperodon systoma. Some idea of the work done on mucosubstances in the gastrointestinal tract of the vertebrates will be obtained from the following brief review:

A) Review of the literature on gastrointestinal tract of the vertebrates.

(a) Esophagus:

The esophagus is a much neglected part of the gastrointestinal tract (Pope et al., 1975). Among the fishes, teleostean esophageal mucous cells have been reported to react with periodic acid Schiff's reaction (PAS) and with Alcian blue (AB) (Bullock, 1967; Bucke, 1971). In a detailed histological and histochemical study, Reifel and Travill (1977) found six types of mucous cells in ten teleost fishes but only two of them were present in each fish. They reported a heterogenous distribution of sialidase resistant sialomucins, sialidase làbile sialomucins, sulfomucins and neutral mucins in mucous cells of esophagi of ten species of teleosts. Chan (1941) suggested that such a presence of two or more carbohydrates in an epithelium could indicate two levels of maturation in the formation of mucus secretion.

In most of the amphibians in which the esophageal glands have been reported, they were shown to be protein or peptic glands. Some of them have also been reported to secrete mucus (Reeder, 1964). These glands were believed by Kingsbury (1894) to be homologous of gastric glands of the stomach and are concentrated in the posterior portion of the esophagus. Norris (1959) reported on PAS positive goblet cells and glands in the esophagus of *Rana pipiens*. Loo and Wong (1975) identified mainly sialomucins, small amount of sulfated mucins and neutral mucins in esophageal goblet cells of the toad, *Bufo melanostictus*.

Varute and Nalavade (1973) studied mucosubstances in epithelium and glands in esophagi of three species of lizards. The epithelium in esophagi of garden lizard (<u>Calotes versicolor</u>) and skink (<u>Mabuya carinata</u>) consisted of only goblet cells which elaborated neutral mucosubstances, sulfomucins and sialomucins. The epithelium in wall lizard (<u>Hemidactylus flavivi-ridis</u>) consisted of goblet cells and columnar epithelial cells, the former cells elaborated mucosubstances similar to the other two lizards, whereas the latter type of cells contained neutral mucosubstances and protein masked sialomucins. The esophageal glands in all the three species of lizards were peptic glands (protein elaborating) and mucous glands (elaborating only neutral mucosubstances). Recently Loo and Swan (1978) demonstrated acidic sulfated mucins in esophageal epithelium of Australian lizard, <u>Egernia cunninghami</u>.

Hanke (1957) described three muscular layers in esophagus wall of two birds, <u>Obsoletus crucis</u> and <u>Tinamus major</u> ; other birds generally contain only two layers of muscles. Protein secreting glands have not been reported in esophagus of birds. In majority of birds, esophageal glands produce acidic mucosubstances which are mainly carboxylated (Grossi and Millo, 1967; Allenspach and Berlin, 1971). On the other hand Van Alten and Fennell (1957) reported on presence of sulfomucins in esophageal glands of birds during histogenesis.

There are sporadic reports in the literature mostly concerned with glycogen in normal esophagus of man and monkey (Wislocki <u>et al</u>., 1951; Rywlin and Ortega, 1970; Themann <u>et al</u>., 1971; Hopwood <u>et al</u>., 1977 a) . Rambourg (1969) reported on the presence of a mucosubstance probably a glycoprotein containing sialic acid between the esophageal cells of rat. Hopwood *et al.* (1977 b) demonstrated neutral mucosubstances in normal human esophageal epithelium. These mucosubstances were observed in the cell coat and membrance coating the granules.

Carvalho *et al.*(1968) identified glycogen,neutral polysaccharides and sialic acid in the mucins of the esophageal glands of armadillo,*Dasypus novemcinctus*. Masuda *et al.*(1977) showed PAS and AB reactive cells in the esophagus of the rabbit and man. The presence of sulfomucins has been reported in the middle layer of esophageal epithelium (Bescol -Liversac and Guillam, 1972) and esophageal glands (Lambert *et al.*,1971). Al Yassin and Toner (1977) considered that the glands in human esophagus can be compared with the mucous secreting minor salivary glands. Sekino and Murata (1978) studied human esophageal acid glycosaminoglycans by electrophoresis and enzyme assays. They reported the presence of hyaluronic acid,dermatan sulfate,heparin sulfate and chondroitin 4- and 6- sulfates which increased with advancing age.

(b) Cardiac Stomach (Corpus or fundic stomach)

Mucosubstances containing in gastric epithelial cells have been reported in many teleosts (Kapoor *et al.*,1975). PAS and AB reactive cells in the gastric mucosa of fish have been reported by Bucke (1971). Recently Reifel and Travill (1978) studied mucosubstances in the surface epithelial cells and cells in gastric pit in stomachs of eight species of teleosts.

They reported a heterogenous distribution of neutral mucosubstances, sialomucins and weakly acidic sulfomucins in stomachs of fishes.

Sedar (1961) studied ultrastructure of Oxyntic cells in gastric glands of the bull frog, R. catesbiana. Norris (1959) demonstrated PAS reactive cells in the surface epithelium and foveolar epithelium in gastric surface of the frog, R.pipiens. The mucous neck cells were also found to exhibit moderate PAS reactivity. Loo and Wong (1975) reported the presence of neutral mucins in columnar cells and neutral mucins and traces of acidic mucins in the mucous neck cells in stomach of the toad (B.melanostictus). Mogil'naya et al.(1978) studied gastric pavement epitheliocytes in amphibians and reported that protection of stomach is connected with neutral carbohydrate components. Leeson (1975) reported that lining cells in the surface pits and gastric pits are columnar mucous secreting in the stomach of the newt, Triturus torosus. These cells differed from mucous neck cells in morphology and staining reactions. Forte and Forte (1970) studied carbohydrates and acid secreting cells of frog stomach. They reported that all cell fractions were extremely low in acidic sugars,uronic acids,sialic acids,while neutral sugars and hexosamine were predominant. The neutral sugars were confined to the membranes. In a later study Beesley and Forte (1973) obtained exceptionally high quantity of carbohydrates from membranes (260 μg/mg protein). Most of the sugars (73%) were found associated with proteins.

Mogil'naya et al. (1978) studied gastric pavement epitheliocytes in reptiles and reported that neutral carbohydrates and sialosaccharides performed the barrier function. Loo and Swan (1978) reported on the presence of neutral mucins in neck cells and both neutral and acidic mucins in the lining epithelial cells in stomach of the Australian lizard, E.cunninghami.

Mogil'naya et al.(1978) reported the presence of neutral carbohydrates, sialosaccharides and sulfosaccharides in gastric epitheliocytes of birds.

Krause and Leeson (1974) studied gastric mucosa of two monotremes,echidna and duckbill platypus. The gastric mucosa was found aglandular and the lining epithelium was stratified squamous type. It consisted of three layers: stratum germinativum, stratum spinosum and stratum corneum. Carvalho et al.(1975) identified proteins rich in α-amino radicals,tryptophan,arginine and sialomucins in gastric lining and pit epithelium,sulfomucins in pit cells and PAS reactive mucous neck cells in the stomach of armadillo, D.novemcinctus. Lambert et al.(1968) demonstrated PAS and AB reactive mucosubstances in surface coat of gastric mucous cells of various animal species including man.

Tsujimura (1976) demonstrated glycogen in neck cells of cardiac stomach,fundic glands and surface epithelium but its absence in the chief and parietal cells of canine stomach. Fruschelli (1967) also reported on significant amount of glycogen in gastric mucosal epithelium in cat,dog and man but absence of glycogen in rat,rabbit,mouse,guinea pig and monkey. Glycogen was found in between the nuclei and upper portion of the cells.

More (1969) reported that mucus secreted by stomach (fundus and antrum) of rabbit contained neutral mucosubstances, whereas Brunner's glands contained acidic mucopolysaccharides. Sheahan and Jervis (1976) studied mucosubstances in gastrointestinal tract of eleven species of mammals such as rat, mouse, hamster, gerbil, guinea pig, rabbit, cat, dog, rhesus, baboon and man. The differences were most obvious in the stomach and duodenum. In each species neutral mucosubstances were most evident in the stomach and acidic mucosubstances in intestines, with sulfomucins most prominent. Acidic mucosubstances were found in the paneth cells of rodents only.

Wattel *et al.* (1977) demonstrated acidic carbohydrates in surface mucous cells and neutral carbohydrates in the mucous neck cells in stomach of rat. Mnatsakanyan (1967) found glycoproteins and increased concentration of sialic acid when a mixture of food is given or after prolonged starvation; there was no change in sialic acid when milk was given. Cuevas-Chavez (1966) by employing a new method known as Hale succinic unhydride, found identical distribution of mucosubstances in gastric glands of diverse animal species. Spicer *et al.* (1978) demonstrated neutral mucosubstances in superficial epithelium, cells in foveòlae, and mucous neck cells in stomach of rat. Sulfomucins were detected only in the foveolar cells.

Tyrkö *et al.* (1968) demonstrated sulfomucins in the human and canine gastric epithelium. Goldman and Ming (1968) in their studies on gastric mucosa described both sulfated and non-sulfated acid mucosubstances. Spicer and Sun (1966) reported the presence of carboxymucins (sialic acid rich) and sulfomucins in gastric epithelium of the dog. Mucous neck cells were found to contain neutral mucins. The zymogen cells abundant in the deep glands of cardiac stomach and body of stomach contained much sulfated mucosubstances. In a later study Spicer et al. (1967 a) confirmed the presence of sulfomucins in the foveolar and pyloric glandular cells in the epithelia and zymogenic cells of dog stomach by autoradiography. Non-sulfated acidic mucosubstances in mucosal area close to the lumen and neutral mucosubstances in neck and parietal cells showed absence of autoradiographic evidence for isotope incorporation in these sites. Woussen-Colle et al. (1975) analyzed glycopeptides derived from glycoproteins present in the mucosa and secretion from fundus and antrum of the dog. Their basic structure was found analogous to the blood group substances but they differed in their fucose, sialic acids and sulfate content. Gerard et al. (1967) reported that the surface epithelium in canine stomach produced glycoproteins and the chief cells and pyloric glands produced chondroitin sulfate A or C. A sialidase susceptible mucin was found predominantly within the superficial cells of the fundus and antrum. The mucous neck cells and parietal cells produced neutral mucosubstances.

Il'in (1971) studied histochemically the mucopolysaccharides in fundic and cardiac stomach of the cattle. The mucopolysaccharides consisted of small amounts of sulfomucins and proteins combined with hyaluronic acid as well as larger component of protein combined with chondroitin sulfate A and C. Roy (1974) demonstrated neutral and acidic mucosubstances in the surface epithelial cells in stomach of domestic pig, Sus scrofa domestica and PAS reactive cells in neck and body of the glands. Sinitsina (1968) showed neutral and acidic sulfated mucins in the superficial epithelium and principal cells in stomach of man. The secretion in auxillary cells represents a peculiar complex of neutral, carboxyl containing and sulfated mucopolysaccharide.

Biochemical assays (Werner, 1953; Schrager, 1964; Schrager and Oates, 1968; Gerard et al., 1967; Martin et al., 1968; Glass et al., 1969), immunological methods (Hakkinen et al., 1968), and autoradiographic studies (Dziewiatkowski, 1956; Jennings and Florey, 1956; Cornet et al., 1964 a,b; Shimamoto et al., 1973) have indicated the presence of sulfoglycoproteins in gastric juice and mucosa of several mammals.

The sulfate containing component, previously isolated from dog gastric secretion by Komarov (1935) may have consisted of one or more of the sulfated mucosubstances secreted by dog gastric zymogenic, foveolar and pyloric glandular epithelia. Hakkinen et al. (1965) isolated four fractions from human gastric juice. None of them contained hexuronic acid but all the fractions contained sulfate in amounts increasing from first to last fraction. Martin et al. (1968) reported fucose, galactose, sialic acid, hexosamine and sulfate (5%) in carbohydrate part of human gastric juice. Mikuni and Hotta (1979) isolated and purified four sulfated glycoproteins from porcine stomach. Kuhn and Weicker (1970) isolated a neuraminic acid rich and fucose containing glycoprotein from gastric juice by phenol water extraction and Sephadex gel filtration.

Waldron (1970) isolated galactose, fucose, N-acetyl glucoseamine, N-acetyl galactosamine and N-acetyl neuraminic acid from human gastric juice. Schrager and Oates (1970) reported on carbohydrate component of glycoprotein from gastric secretion which consisted of galactose, glucosamine and galactosamine in the ratio - 4:3:1. Oates *et al.* (1977) reported the presence of amino acids and sugars such as N-acetyl galactosamine, N-acetyl glucosamine and galactose in ratio of 1:3:4. Woussen-colle *et al.* (1975) found that the fundic mucus in dog contained mainly acidic glycoproteins with predominance of sulfated glycoproteins, whereas antral mucus was almost exclusively of neutral glycoproteins and sialoglycoproteins.

Pallavicini *et al.* (1971) isolated 3 glycopeptide fractions from pig gastric mucins with differing degree of acidity. Analysis by chromatography on Dowex 1 showed the presence of sialic acid and mannose on terminal positions and galactosamine on internal positions. Schrager (1974) studied glycoproteins from gastric and intestinal mucus. These include 75% to 80% carbohydrates, the nature of which resembled to that of the blood group substances. By electrophoretic separation De Grafe and Glass (1968) characterized sulfated glycoproteins of dog stomach secretions. Their studies revealed the presence of chondroitin sulfate A and sulfated glycoprotein of intermediate charge containing hexosamine, hexose, fucose, sialic acid and sulfates in gastric juice.

(c) Pyloric stomach

Reifel and Travill (1978) studied carbohydrates in pyloric epithelial cells in stomach of eight species of teleosts and reported a heterogenous distribution of sialidase resistant sialomucins, sialidase labile sialomucins, weakly acidic sulfomucins and neutral mucosubstances. Norris (1959) reported that the epithelial cells and pyloric gland cells in frog, *R. pipiens* exhibited PAS positive reaction. The bird stomach is a dual structure. The anterior proventriculus is glandular and functions in food storage, protein digestion, fat digestion and HCl secretion (Patt and Patt, 1969). The posterior region, the ventriculus or gizzard is involved in food grinding. In few birds such as heron, stork and cormorant, a third section, pyloric stomach, is evident. Mogil'naya and Bogatyr (1977) demonstrated histochemically the presence of neutral polysaccharides, sulfo-and sialomucopolysaccharides in the mucous secretion of the avian gizzard.

Carvalho et al. (1973) demonstrated neutral mucopolysaccharides associated with carboxyl groups in pyloric glands of armadillo. The surface epithelium in pyloric stomach contained sulfated and carboxyl containing mucopolysaccharides, whereas the cells in pit contained neutral mucopolysaccharides associated with sulfate groups. Birgele (1969) reported on neutral mucopolysaccharides in epithelial cells, neutral and acidic mucopolysaccharides in pyloric glands in abomasum of cow. Il'in (1971) showed that the secretions of the pyloric stomach in cattle contained proteins with - SH groups and small amount of protein complexed with sulfated mucopolysaccharides. Poddar and Jacob (1979) demonstrated neutral mucosubstances in the pyloric glands of ferret, the superficial cells contained sialidase labile and resistant sialomucins. Tsujimura (1976) identified small amount of glycogen in pyloric glands of dog. Reactions to the neutral mucopolysaccharides were stronger in pyloric glands than in the chief cells in fundic stomach but reactions to sulfomucins and sialomucins were weaker than in the chief cells.

(d) Duodenum

Krause (1973) studied mucosubstances in the duodenal glands in six marsupial species. The duodenal glands in kangaroo, native cat, marsupial mouse and bandicoot formed a collar, distal to gastrointestinal junction and elaborated neutral mucosubstances, whereas these glands were diffusely scattered along intestinal tract in koala and wombat which elaborated acidic mucosubstances. In rabbit, the duodenal glands contain two distinct cell types, serous and mucous (Leeson and Leeson,1967). More and Bayle(1972) identified sulfomucins in duodenal caliciform cells and sialic acid in light cells of Brunner's glands in rabbit. Uspenskii (1972) also demonstrated sialomucins and sulfomucins in the goblet cells of duodenum.

Poddar and Jacob (1979) reported that the duodenal goblet cells in ferret show variations in their histochemical characters. Some of the deep cells and majority of the superficial cells contain sialidase labile and sialidase resistant sialomucins, whereas few superficial cells and occasional deep cells contain sulfated mucosubstances. The presence of sulfomucins has been confirmed in the Brunner's glands of guinea pig (Jennings and Florey, 1956) and duodenal mucosa of man (Hoskins and Zamcheck, 1963). Silva et al.(1973) demonstrated neutral mucopolysaccharides together with sulfated mucopolysaccharides in mucosal goblet cells in cat.

Carvalho et al.(1972) reported the presence of neutral mucopolysaccharides, carboxylic acid containing mucins and sulfomucins in the duodenal(Brunner's)glands of the goat, Capra hircus; the goblet cells contained only sialomucins. On the other hand

Sinitsina (1966) demonstrated neutral mucopolysaccharides, carboxymucins, sialomucins and sulfated mucopolysaccharides in goblet cells and neutral mucopolysaccharides in the paneth cells and Brunner's glands of human duodenum. Shackleford and Wilborn (1978) described sexual dimorphism in duodenal glands of hamsters, wherein the males contained double the amount of acidic mucosubstances than the females and PAS reactivity was stronger in the duodenal glands of the females than the males.

Koshevaya (1972) studied the composition of the duodenal secretions of young calf. The separated elements were identified as polysaccharides, glycolipids, glycogen, sialic acid and hyaluronidase resistant chondroitin type of sulfate.

(e) Small intestine (Ileum)

Bucke (1971) reported the presence of PAS and AB reactive cells in the intestinal epithelium of the fish *E. lucius*. Reifel and Travill (1979) studied mucosubstances in intestine of ten species of teleosts. Their histochemical results revealed the presence of sialidase resistant sialomucins and some weakly acidic sulfomucins in the mucous cells in *A. rupestris*, *L. macrochirus*, *M. salmoides*, *P. nigromaculatus*, *N. crysoleucas*, *P. promelas*, *I. nebulosus* and *P. flavescens*. The mucous cells in *E. americanus* contained only sialomucins, whereas those in *E. luceus* contained sialomucins and neutral mucosubstances.

Mc Avoy and Dixon (1978) showed the presence of columnar cells and goblet cells in the small intestine of toads, *Xenopus laevis*. Loo and Wong (1975) demonstrated sulfated mucins in the goblet cells and granules in the columnar cells in the small intestine of toads, B.melanostictus.Lestage (1973) reported that in the anterior intestine of the toad, B.bufo larva,starch rich and lipid rich diets caused increase in number of mucous cells. A lipid rich diet favoured secretion of acidic mucins.(Predominantly sialomucins), whereas starch rich diet favoured secretion of neutral mucins.

Anwar and Mohmoud (1975) reported the presence of goblet cells in the ileum of two Egyptian lizards,Mabuya quinque taeniata and Chalcides ocellatus which elaborated acidic mucoproteins. Loo and Swan (1978) identified acidic sulfated mucins in the intestine of Australiun lizard, E.cunninghami.

Mnatsakanyan (1967) reported an increase in glycoproteins and sialic acid in intestine of the rat when a mixture of food is given or after prolonged starvation. Poddar and Jacob(1979) demonstrated goblet cells in the villi and crypts in the intestine of ferret, the secretions of which contained a mixture of sulfomucins,sialidase resistant sialomucins and sialidase labile sialomucins. Hecker (1973) reported on soluble mucins in the gastrointestinal tract of sheep. The mucins were soluble in the liquor from large intestinal contents. Skorodinskii et al.(1970) demonstrated neutral mucopolysaccharides in the goblet cells of intestinal villi and crypts as well as in peeled off cells in the intestinal tract of cattle and sheep.

Lev and Spicer (1965) reported the presence of PAS reactive, sialic acid containing and sulfated epithelial mucins in the human intestine. Hauri et al.(1977) showed C^{14} glucosamine incorporation in brush border glycoproteins of human intestinal mucosa.

Bescol-Liversac and Guillam (1972) demonstrated sulfomucin synthesis in intestinal caliciform cells by autoradiographic studies. Filipe and Fenger (1979) demonstrated neutral mucins and sialomucins in the goblet cells of villi in normal small intestine and benign tumors in intestine. The mucosa adjacent to carcinoma showed increasing amounts of sialomucins and sulfomucins. Subbuswamy (1971) earlier detected predominantly neutral mucins in the goblet cells of human intestine. Kim (1972) studied small intestine and large intestine of 7-mammals, 8-birds, 6-reptiles, 5-amphibians and 7-fishes. The mucins in the goblet cells in them contained neutral and acidic (sulfated) mucopolysaccharides. They varied according to species and regions they inhabited.

Chromatographic isolation studies of mucins from small intestine of the pig by Degand *et al.*(1972)revealed 16% sialomucins and 78% sulfomucins. Srinivas *et al.*(1971) also isolated acid mucopolysacchrides from mucosal scrapings of pig intestine after proteolytic digestion and anion exchange chromatography. The isolated mucopolysaccharides contained equimolar proportion of uronic acid,hexosamine and sulfate (about 25%).

(f) Large intestine

Reifel and Travill (1979) studied mucosubstances in rectal or distal intestine of ten teleost fishes. They reported the presence of sialidase resistant sialomucins and weakly acidic sulfomucins in mucous cells in distal intestine of *A.rupestris*, *L.macrochirus*, *M.salmoides*, *P.nigromaculatus*, *N.crysoleucas*,*P. promelas*, *E.americanus* and *P.flavescens*,only neutral mucosubs-

-tances in E.lucius and only sulfomucins in I.nebulosus. Subbuswamy (1971) demonstrated acid mucins in the goblet cells of human large intestine. Masuda et al(1977) reported that the mucosa of the large intestine in rabbit and man contained PAS and AB reactive glycoproteins. Kim (1972) identified neutral and acidic sulfated mucopolysaccharides in the goblet cells of large intestine of 7-mammals,8-birds, 6-reptiles, 5-amphibians and 7-fishes. Some variations were also noted according to the species and regions where the goblet cells present.

(g) Cloaca

Loo and Wong (1975) demonstrated mainly sulfated mucins and small amount of neutral mucins in the goblet cells and granules in the columnar epithelial cells of the cloaca of the toad,B.melanostictus. In another study Loo and Swan (1978) identified only sulfated mucins in the mucosal epithelium of the cloaca of the Australian lizard, E.cunninghami.

(h) Colon

Biochemical studies by Kent (1963) revealed the presence of sulfated sialoprotein in the colonic mucins of the sheep. In another study Kent and Draper(1968) identified N-acetyl and N-glycollil neuraminic acid and a third to be diacetylated neuraminic acid in the colonic mucins of the sheep.Culling and Reid (1979) also confirmed the presence of sialic acid in colonic mucins of rat and man. Filipe and Branfoot(1974) also reported that sialomucins replaced sulfomucins as the principal mucosubstances in the goblet cells of human colon. Earlier, Inoue and Yosizawa(1966) isolated and purified mucopolysaccharides from pig colonic mucosa.

They were grouped into two types: sulfated sialopolysaccharides and sulfated uronic acid containing mucopolysaccharides.

(i) Rectum

Chemical analysis of the carbohydrate components of human rectal mucus showed larger amounts of sialomucins and lesser amount of fucomucin (Hoskins and Zamcheck,1963). Masuda et al. (1977) reported that glycoprotein content was greater in mucosa than serosa of rabbit and man, and it was highest in mucosa of the rectum. The glycoproteins were PAS and AB reactive.

B) 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 vertebrate alimentary tract.The literature on animal mucosubstances significantly shows that:

I)Though the various tissues and organ-systems such as connective tissue, cartilage,respiratory tract,reproductive tract, kidney,brain and nervous system,muscles,major salivary glands, lingual glands etc. 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 our status of the knowledge on mucosubstances in the vertebrate 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 submammalian vertebrates.

III)Whatever work has been reported on the mucosubstances in the vertebrate alimentary tract, some investigators have selected one organ of a given animal others have selected altogether a different organ of a different animal. 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.

IV)There is also scanty information available on sexual dimorphism of duodenal mucosubstances (Shackleford and Wilborn, 1978) but no information is available on sexual dimorphism of alimentary tract mucosubstances in other animals.

V) In some cases only PAS reactivity or AB reactivity of a given cell type in mucosa or the gland has been reported but the mucosubstances have not been further identified.

VI) Recently Loo and Wong (1975) have rightly pointed out that there is very little work published on the mucins of the gastrointestinal tract of the Anurans.

The above critical evaluation of the existing literature on vertebrate alimentary tract mucosubstances makes it very clear that a detailed investigation of mucosubstances in vertebrate alimentary tract is essential to augment their nature and role played in the physiology of the alimentary tract and also to augment the understanding of animal mucosubstances in general. It is with this view, therefore, that an extensive research project has been undertaken in this laboratory to study the mucosubstances in various organs of the alimentary tract of different vertebrates, i.e. from fishes to mammals. When the entire research project will be completed, most of the lacunae in our present day understanding of vertebrate alimentary tract mucosubstances will be satisfactorily removed. The work included in the present thesis forms a part of this extensive research scheme and concerns with the mucosubstances, their histochemical characterization and distribution in various organs of the alimentary tract of the frog, E.systoma. A battery of recently developed and recommended histochemical techniques has been employed to achieve a technical and methodological perfection.

C) Plan of the present investigation.

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

a) Choice of the animal

While selecting the animal care was taken to select such an amphibian wherein no work has been carried out on mucosubstances in any organ of the alimentary tract. Secondly the animals both males and females should be available in required number throughout the course of the investigation. Therefore, the frog, E.systoma was found most suitable for the present investigation.

b) Choice of the techniques

Since the present investigation aims at a detailed study

(1) Histology of the various organs such as esophagus, stomach (cardiac and pyloric), duodenum, small intestine and large intestine.

(2) Sex dimorphism, if any, in the various organs in male and female frogs.

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

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

(5) To compare the results obtained in the present investigation and the existing literature on other amphibians so as to find out similarities or differences, if any.

(6) To compare the results obtained in the present investigation and existing literature on vertebrate alimentary tract to find out any phylogenetic variations.

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

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

d) <u>Presentation of the thesis</u>

It was decided to divide the present thesis into four chapters, the first chapter being on the introduction, it gives a brief review of the existing literature on mucosubstances in the various organs of the alimentary tract of vertebrates, 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 esophagus to large intestine of the frog under present investigation are listed in chapter III. The chapter four is devoted to the discussion on 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 thesis will be given at the end of the thesis.